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BORON DEFICIENCY IN GARDEN AND SUGAR BEET[1]

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INTRODUCTION

The heart and dry rot of mangel and sugar beet (*Beta vulgaris* L.) was shown by Brandenburg (*5*)[2] in 1931 to be due to a deficiency in available boron. This work has been confirmed by numerous investigators, and much of the recent literature on the subject has been reviewed by Dennis et al. (*9, 10, 11*) and Brandenburg (*6*). The internal black spot of garden beet has become increasingly serious during the past 10 years in various parts of the United States, particularly in those areas in the Northern States where the crop is grown for canning. This disease has already been described (*21*) and the symptoms, therefore, need not be reviewed here. In previous publications (*1, 21*) the possibility of the relation of boron deficiency to this malady was pointed out. About the same time similar indications were secured in New York (*20*), and more recently confirmatory results were published from Oregon,[3] Michigan (*7*), and New York (*19*). The present paper is a report of investigations which have been under way in Wisconsin since 1937 and of which several preliminary reports have been made (*1, 21, 22, 23, 24*).

METHODS AND MATERIALS

In order to study critically the relation of boron to the development of the garden beet, controlled nutrient experiments were conducted in quartz-sand cultures in the greenhouse. An adaptation of the continuous-drip method of Allison and Shive (*2*) was used. The standard complete nutrient solution was a modification of Shive's Best three-salt solution.[4] It was diluted with 10 parts of distilled water for use in the drip system, and a continuous flow of air was provided in the reservoir containing the solution. Clay pots used to contain the quartz sand were varnished before filling; boronfree glassware and as pure chemicals as were available were employed.[5] The complete solution when diluted contained approximately 0.75 p.p.m. of boron. The boronfree solution was similar in every way except for the omission of the boric acid. Seed was sown directly in the sand, being supplied with either the complete or the boronfree nutrient. When the effect of change from the complete to the deficient solution and vice versa was to be studied, all plants were first removed

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[2] Italic numbers in parentheses refer to Literature Cited, p. 122.

[3] POWERS, W. L., and BOUQUET, A. G. B. USE OF BORON IN CONTROLLING CANKER OF TABLE BEETS. Oreg. Agr. Expt. Sta. Cir. of Inform. 195, 6 pp., illus. 1939. [Processed.]

[4] The formula used was as follows: KH_2PO_4, 0.018 M; $Ca(NO_3)_2.4H_2O$, 0.0052 M; $MgSO_4.7H_2O$, 0.015 M; traces of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_3.

[5] The writers are indebted to Dr. Kermit C. Berger, Department of Soils, for testing the chemicals and the water supply for the presence of boron.

from the "complete" and "boronfree" pots. A certain number were then transplanted to the original pots and the remaining ones were exchanged and then transplanted. Similar handling of all plants was thus obtained. When the effect of boron starvation at later stages was to be studied, plants were grown to the desired stage in the complete nutrient solution and the entire pot was then transferred to the boron-deficient nutrient solution.

Field studies were carried out in several locations in Wisconsin. Land for this purpose was usually selected in fields which had shown heart rot of sugar beet or internal black spot of garden beet in previous crops. In determining the extent of internal black spot of garden beet in the field experiments two criteria were used. Random samples of 25 or 50 plants were taken from each treatment plot. Each root was cut in slices approximately one-quarter inch in thickness and each piece examined for the disease. The percentage of plants showing any sign of disease was recorded as the first criterion. The beets were, in addition, placed in one or another of four classes which represented grades of disease: i.e., clean, slight, moderate, severe. By giving these classes specified weights a disease index was calculated which constituted the second criterion. The weights given to the different classes were as follows: Clean, 0; slight, 20; moderate, 50; severe, 100. To secure the disease index, the number of plants in each class was multiplied by the class weight and the sum of these figures was divided by the product of the total number of plants.

Boron was applied as commercial borax,[6] usually incorporated in the fertilizer. This mixture was applied either broadcast or in drills in a separate furrow 1½ to 2 inches at one or both sides of the row and slightly deeper than the seed. Broadcast applications were made with a fertilizer sower and the materials were disked into the soil immediately.

EXPERIMENTAL RESULTS

GREENHOUSE STUDIES

Plants in Sand-Nutrient Cultures

Seedlings appeared 4 to 6 days after seed was sown in sand cultures. When the nutrient solution was free from boron from the beginning, deficiency symptoms appeared promptly on the young unfolding leaves. An intensification of the red pigment was commonly perceptible first along the midrib and veins and then at the tip or on one side of the leaf. Some unilateral development occurred, and reduction in growth rate was apparent. In figure 1, seedlings of two varieties, Detroit Dark Red (Ferry strain) and Good For All, are shown 31 days after sowing in normal and in boron-deficient cultures. Stunting was much more pronounced in the Good For All variety, which is in conformity with its greater susceptibility in the field to be described later. If boron-starved plants were continued longer in boronfree nutrient, growth was very slow and the plants changed relatively little in appearance, although a large percentage of them died in the course of several weeks.

When plants grown for 30 to 40 days in normal and boron-deficient solutions were interchanged, acute symptoms soon appeared in the

[6] Most of the borax for these experiments was supplied by the Pacific Coast Borax Co.

healthy plants while the deficient plants recovered rapidly. This was shown in the same two varieties in figure 2. In the Detroit Dark

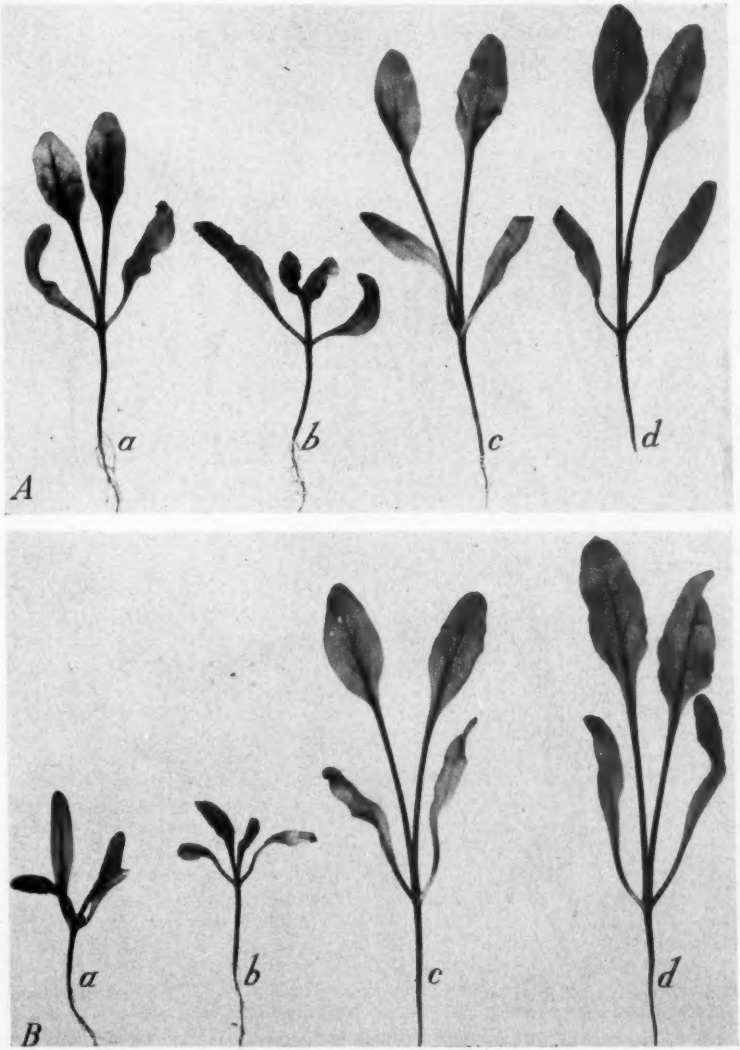

FIGURE 1.—Beets grown from seed in sand-nutrient cultures for 31 days. *A* Detroit Dark Red variety (Ferry strain); *B*, Good For All variety. In both *A* and *B*: *a*, *b*, plants grown in boronfree nutrient; *c*, *d*, plants grown in complete solution.

Red plant started in normal solution and transferred to the deficient solution (fig. 2, *A*, *c*) the youngest leaves remained dwarfed and the growing tip died, although little change but that of deeper red color

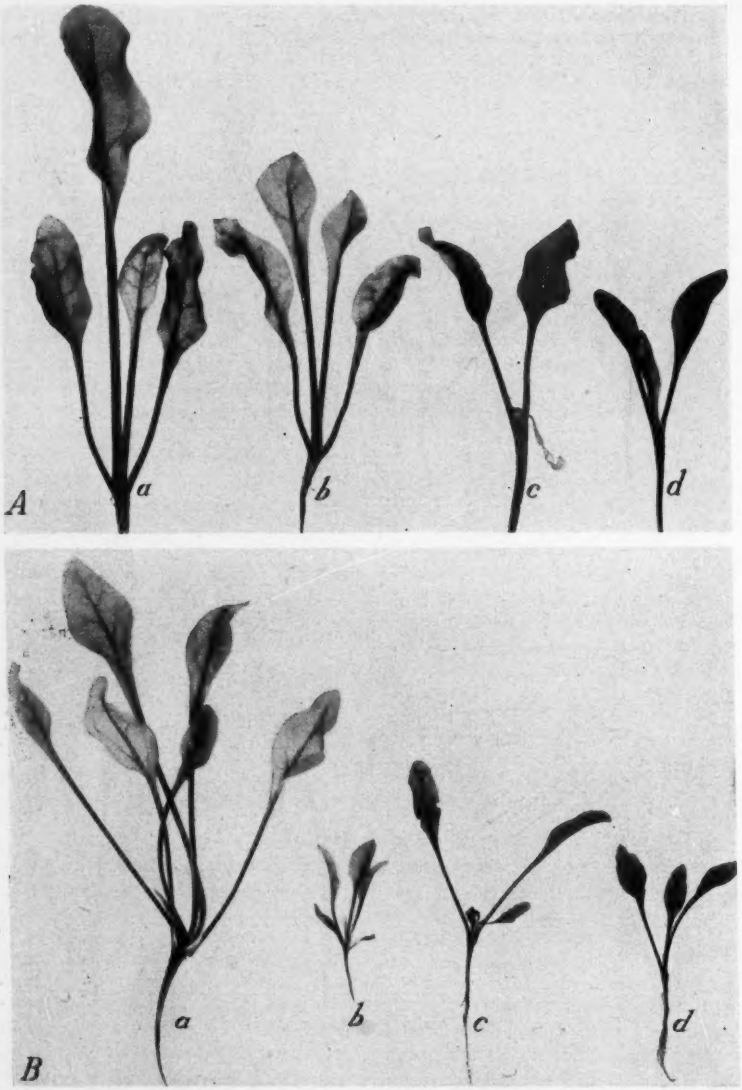

FIGURE 2.—Beets grown in sand cultures in complete solution for 38 days and then transferred to boronfree nutrient for 22 days, and vice versa. *A*, Detroit Dark Red variety (Ferry strain); *B*, Good For All variety. In both *A* and *B*: *a*, plant grown in complete solution for 60 days; *b*, plant grown in boronfree solution for 38 days, then in complete solution for 22 days; *c*, plant grown in complete solution for 38 days, then in boronfree solution for 22 days; *d*, plant grown in boron-deficient solution for 60 days.

occurred in the first two leaves, which were nearly mature when the transfer was made. This behavior is in contrast with that of the plant grown continuously in boron-deficient nutrient (fig. 2, *A*, *d*) which was dwarfed early and, growing more slowly, required less boron than the one growing vigorously in the normal solution when it was suddenly deprived of this element (fig. 2, *A*, *c*). It would appear that the boron acquired by the first two leaves in this plant was rapidly fixed and as already reported by Brandenburg (*6*), was not subject to translocation to the growing point. The plant grown in boron-deficient solution for 38 days (fig. 2, *B*, *d*) had its original meristem intact and consequently a plant similarly treated (fig. 2, *A*, *b*) commenced normal growth when transferred to the normal solution.

The response of Good For All plants was of the same order as that of Detroit Dark Red. However, the effect of boron-deficiency was greater at 38 days in this more susceptible variety (fig. 2, *B*, *d*). The recovery of the deficient plant was less rapid (fig. 2, *B*, *b*) and the collapse of the plant transferred from normal to deficient solution was more prompt (fig. 2, *B*, *c*).

When plants were allowed to attain greater size in the normal solution before boronfree nutrient was applied or when a trace of boron was left in the nutrient, symptoms developed more slowly and the disease picture was somewhat more suggestive of the usual appearance of the disease in the field. In figure 3 are two plants grown for 17 days from seed in the complete solution, then watered with a reduced-boron nutrient. Boron-deficiency symptoms did not appear until 43 days later, and the photograph was taken after another 50 days had elapsed. In figure 3, *A*, the first three leaves were normal in size and color although chlorosis in the oldest leaves sometimes occurred. The next two leaves were normal in size but the red pigment was intensified somewhat, while the next younger leaves showed malformation typical of boron deficiency. In figure 3, *B*, the first four leaves were normal, the next two were somewhat deeper in color, while the seventh leaf showed typical unilateral malformation. The next younger leaves appeared quite normal, possibly as a result of the release of boron from dying leaves and roots which had become incorporated in the sand.

When beet plants were grown in the complete solution long enough to permit taproot enlargement, boron starvation brought on necrosis, usually near the periphery, in the rings in which bundles had been differentiated most recently. Sometimes, however, extensive darkening was produced throughout the center of the beet. In figure 4 are the cross sections of roots of two plants grown in nutrient-sand cultures for 145 days. The larger root, free from necrosis, was grown in complete solution throughout the period. The smaller root was grown on complete solution for 71 days, then in boronfree nutrient for 74 days. The black corky tissue is not unlike that found in beet roots affected with internal black spot in nature.

Beet roots showing typical internal black spot were collected in the field and halved longitudinally. Corresponding halves were placed in normal and in boron-deficient nutrient in sand cultures. Extensive development of normal leaves ensued in the former, although no change in the necrotic areas of the fleshy root occurred. In the latter, stunted malformed leaves formed and died, only to be followed by others arising from dormant buds at the bases of the

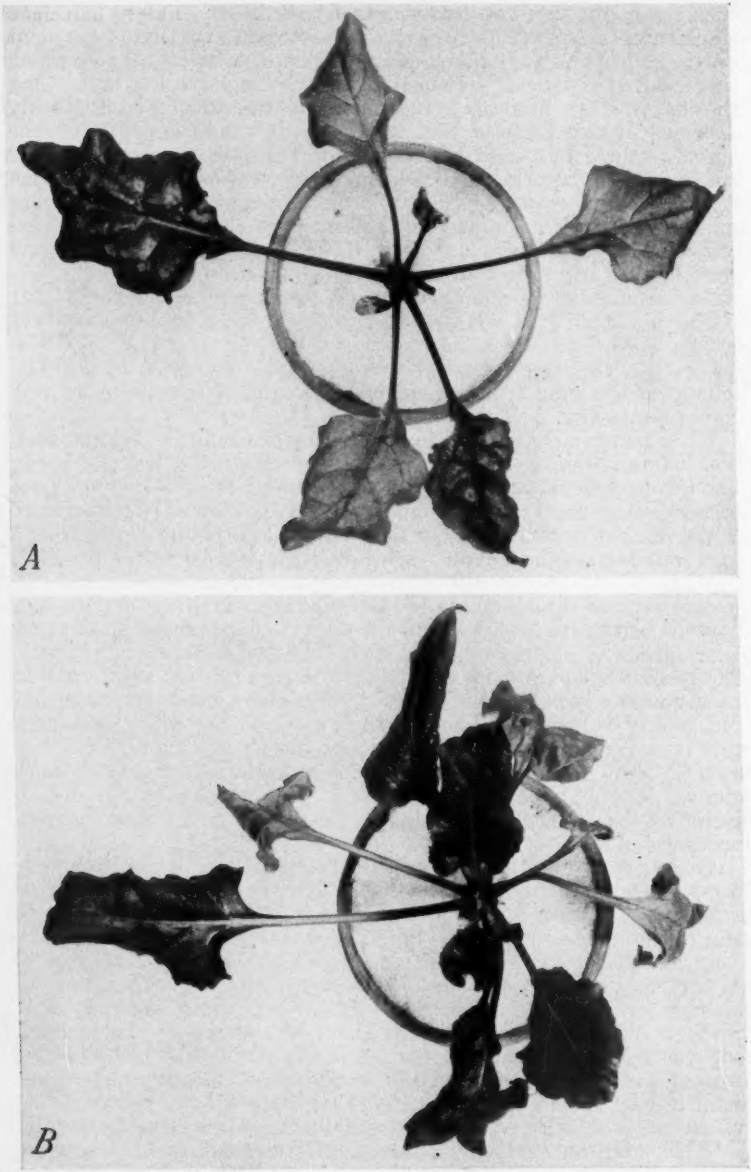

FIGURE 3.—Beet plants of Detroit Dark Red variety (Ferry strain) grown from seed for 17 days in sand culture with complete nutrient solution, then with a solution containing traces of boron for 93 days. See further explanation in the text.

original ones, leading eventually to a rosette of malformed and dead leaves. Such a pair of plants is shown in figure 5.

When beet roots grown in the field with an adequate supply of boron were planted after a period of dormancy, in boronfree nutrient sand cultures, leaf and floral stem development was at first normal, but eventually the apical leaves showed symptoms of boron

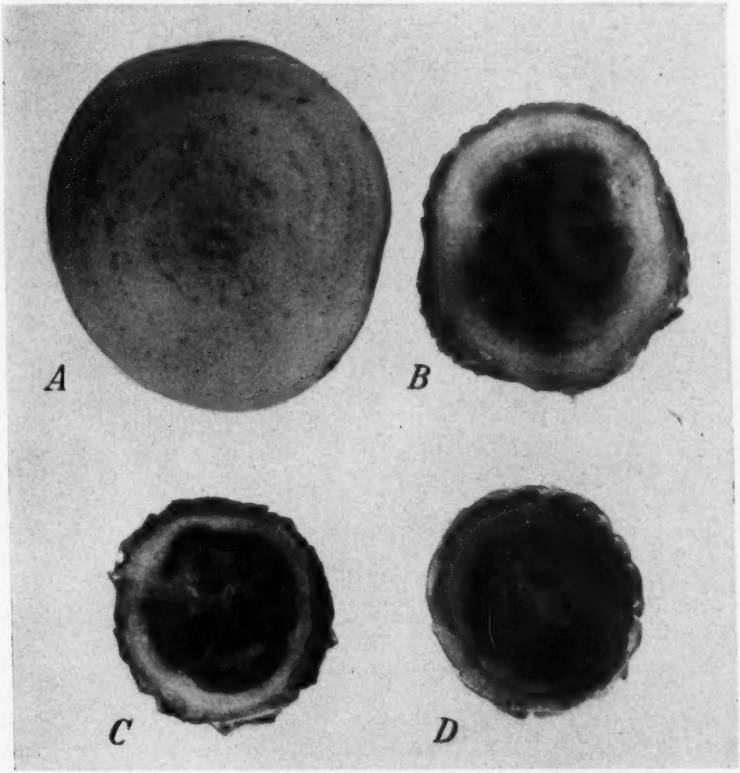

FIGURE 4.—*A*, Cross section of the root of a beet plant grown from seed in complete-nutrient sand culture for 145 days. *B*, *C*, *D*, Cross sections of the roots of beet plants grown concurrently with that in *A* in complete solution for 71 days, then in boronfree nutrient for 74 days.

deficiency. Frequently elongate fissures developed in the stem, sometimes extending its full length. Gradual reduction in growth generally followed. These phases of the disease are illustrated in figure 6.

PLANTS IN SOIL

The appearance of symptoms of boron deficiency in garden beets grown in sand-nutrient cultures was compared with that of plants grown in the greenhouse in the winter months at Madison, Wis., on soil collected from fields in which severe internal black spot had occurred. It was at first postulated that a simple greenhouse pot

test might be devised in which the relative availability of boron in the soil concerned might be estimated as a guide to field requirements

FIGURE 5.—A beet root showing internal black spot when collected in the field at maturity was split longitudinally and one part (*A*) was placed in sand culture with a boronfree nutrient, while the other (*B*) was placed in sand culture with a complete solution. Photographed 87 days later. See further explanation in the text.

for correction of the deficiency. In general the acute boron-deficiency symptoms described above in the sand-nutrient cultures were absent,

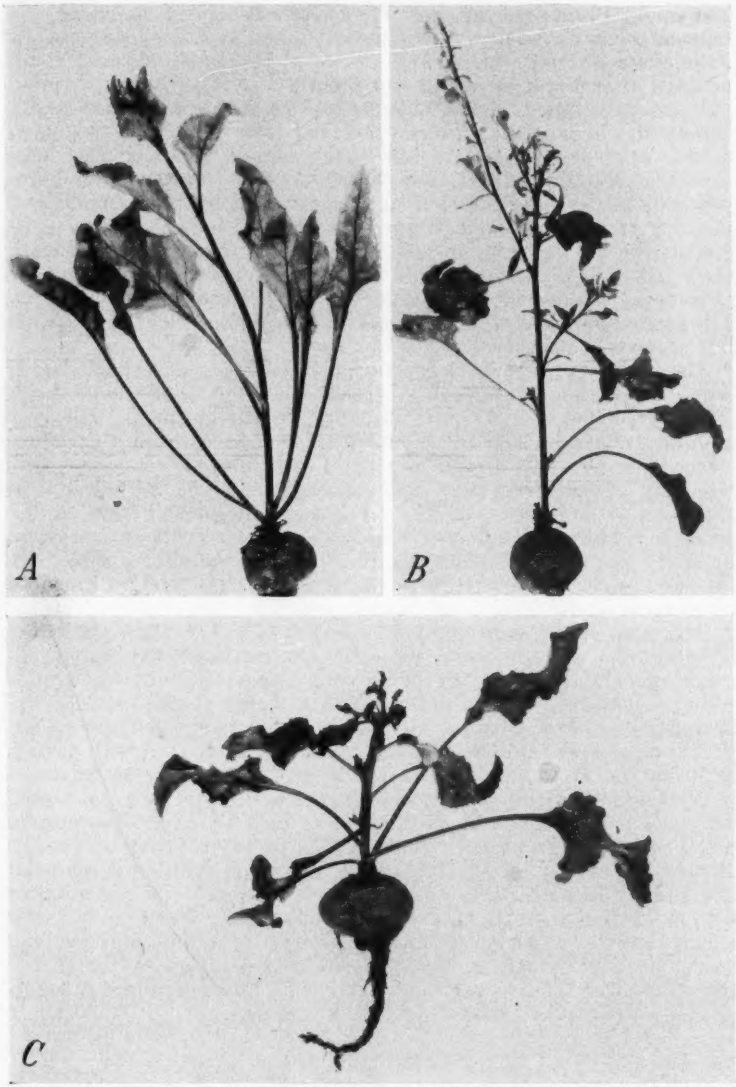

FIGURE 6.—Beets grown in the field with an adequate boron supply, stored at 5° C. for 60 days, planted in sand-nutrient cultures and photographed 60 days later. *A*, plant grown in complete nutrient; *B*, *C*, plants grown in boronfree nutrient. Note advanced flower development in *B* and *C*, stunting of terminal bud in *B* with consequent development of floral stems from leaf axes, and stunting and elongate stem cankers in *C*.

except for somewhat greater intensification of red pigment and reduced vigor. Unilateral leaf development, cross hatching of the petiole, apical bud necrosis, and rosette of the tops appeared rarely or only after a period of several months. When various amounts of borax were added to such soils the growth of the plants was usually enhanced and normal color of the foliage returned. The results of many such comparisons indicated that soils in which garden beets were severely affected were not completely free from available boron but sufficiently so at the time of greatest need by the growing crop to bring on deficiency symptoms. It was also indicated that under greenhouse management of soil where moisture was more uniform, there was not the extreme development of boron-deficiency symptoms as sometimes observed in the same soil under certain field conditions. The plant symptoms which resulted were therefore those of moderate but protracted deficiency.

The symptoms of boron deficiency in garden beets in the field already described (*21*) may be regarded as the combination of effects produced in the greenhouse in soil and in sand-nutrient cultures. The crop planted on boron-deficient soil in Wisconsin in May or early June grows quite normally insofar as general appearance is concerned until late July or early August. Some intensification of red pigment or yellowing of older leaves may occur, but this may result from various other environmental influences and it does not serve as a consistent means of diagnosis. In August and September the disease appears in tops and roots, the extent and severity again being greatly influenced by climatic environment. If the soil is acutely deficient in boron the greater need of the enlarging plants results in the regular appearance of symptoms. If the boron supply is marginal, distinguishable top symptoms may fail to appear or they may consist only of slight interveinal yellowing. On the other hand, in marginal cases sudden appearance of extensive and acute leaf and root symptoms may occur. This type of disease development is commonly associated with periods of drought. However, the writers' observations have shown that heavy rainfall starting sudden new growth activity after a protracted dry spell is probably the most effective incitant of severe internal black spot. It is possible that during protracted dry spells the already low supply of available boron in the soil solution is rendered temporarily less available and that concomitantly the fibrous root system of the plant is rendered less effective. When under such circumstances suddenly stimulated growth of plants approaching maximum development is incited by an abundant water supply, the symptoms which follow are most nearly like those produced in sand-nutrient cultures by extreme deficiency of boron.

FIELD STUDIES

Certain preliminary field experiments in 1937 have already been reported (*21*). These were side-row applications made when the plants were in the third- or fourth-leaf stage. They indicated a tendency for black spot to be reduced, although in none of the fields used in this series was the disease very severe. In the late summer of 1937 a limited survey was made of fields of garden beet in Wisconsin in which black spot was present. Other surveys were made in 1938, 1939, and 1940. The results are summarized in table 1. More than

60 percent of the fields examined critically during this 4-year period were neutral or alkaline in soil reaction, but more than 15 percent were within the range of pH 5.2 to 5.5. About the same number of fields in which the disease was rated as severe were in low areas as in high upland tracts. It had been already noted in 1937 (*21*) that no

TABLE 1.—*The occurrence of internal black spot of garden beets in certain commercial fields in Wisconsin, 1937–40*

Field No.	Kind of soil	Topography of portion of field examined	pH of soil [1]	Severity of disease	Year
1	Light sandy loam	Hillside	8.0	Medium	1938
2	Light silt loam	Level upland	8.0	do	1938
3	Dark sandy loam	do	8.0	Severe	1938
4	Dark loam	Lowland	8.0	Medium	1938
5	do	do	8.0	Severe	1938
6	Light loam	Hillside	8.0	do	1938
7	do	do	8.0	do	1938
8	Dark silt loam	Lowland	8.0	do	1939
9	Light sandy loam	Level upland	8.0	do	1939
10	Dark silty clay loam	Low	7.9	do	1937
11			7.9	do	1937
12		High and low	7.8	do	1937
13	Dark silt loam	Low	7.8	do	1937
14	do	do	7.7	do	1937
15	Medium fine sandy loam	High	7.5	Slight	1938
16	Dark silt loam	Low	7.5	Severe	1939
17	do	Level upland	7.5	do	1940
18	Medium silt loam	Upland	7.5	do	1940
19	Dark silt loam	Swale	7.2	do	1939
20	Medium silt loam	Hillside	7.2	do	1939
21	Light sandy loam	Level upland	7.2	do	1939
22	Light fine sandy loam	Hillside	7.0	Medium	1938
23	do	Hilltop	7.0	do	1938
24	Dark silt loam	Low	7.0	Severe	1939
25	do	do	7.0	do	1940
26	do	Hillside	6.8	do	1939
27	do	Low	6.7	do	1939
28	Light sandy loam	Level upland	6.5	do	1938
29	Peat	Low	6.3	do	1939
30	Medium silt loam	Hillside	6.2	Medium	1937
31	Medium sandy loam	Level upland	6.2	Severe	1939
32	Light silty loam	do	6.1	do	1938
33	Medium silt loam	Low	6.0	Medium	1937
34		High	5.5	Severe	1937
35		do	5.5	Medium	1937
36	Light sandy loam	Level upland	5.5	do	1940
37	do	do	5.5	Severe	1940
38	do	Hillside	5.4	do	1939
39	do	Hillside and hilltop	5.4	do	1940
40	do	Level upland	5.2	Medium	1940

[1] The determinations of soil reaction and kind of soil were kindly made by Dr. Harold H. Hull, Department of Soils, University of Wisconsin.

correlation existed between the occurrence of internal black spot of garden beet and the topography of the soil, and this was borne out when the survey was extended 3 years longer. While a preponderance of the disease occurs on neutral or alkaline soil, it is not necessarily restricted to such soils in Wisconsin.

FIELD EXPERIMENTS AT WINNECONNE, WIS.

The field used at Winneconne, Wis., consisted of poorly drained Poygan silty clay loam, relatively high in organic matter and for the most part slightly alkaline in reaction. Broadcast applications were made in the spring of 1938 on a series of plots in this field, and the disease reaction on beets was studied in three successive seasons, 1938, 1939, 1940. Side-of-row applications were made in another series of row plots. Since manganese deficiency was suspected in this soil, application of manganese sulphate was made in some plots alone and

with borax. The salts of various other minor elements were also included to determine any possible influence they might have on the development of the disease. The results of broadcast applications will be discussed first.

Broadcast applications.—Plots 30 by 30 feet in dimension were laid out in the spring of 1938 in four replicate series of nine treatments each, the latter being arranged in random order and each plot surrounded by a 5-foot untreated border.[7] Treatments were made about 2 weeks before sowing. In the spring of 1939 each plot was divided into two equal parts on one of which the treatment of 1938 was repeated. In the spring of 1940 no minor-element treatments were made but a complete fertilizer was applied broadcast just before sowing. In 1938 and 1939 sugar beets, garden beets and Wisconsin Refugee beans (*Phaseolus vulgaris* L.) were planted in each plot. The last species was used because it is regarded as rather sensitive to boron toxicity and it thus served as an index of any detrimental effects of treatment. Cabbage (*Brassica oleracea capitata* L.), cauliflower (*B. oleracea botrytis* L.), and certain other crucifers were also included; the studies with these crops are reported elsewhere (*25*). In the case of garden beet the two varieties Good For All and Detroit Dark Red were used. The same seed stock of the former was planted in 1938 and 1939. Although seed of the second variety was secured each season from the Ferry-Morse Seed Co. of Detroit, Mich., it was later realized that the so-called Ferry strain of Detroit Dark Red was used in 1938 and the Morse strain of that variety in 1939. It was also learned later that the Morse strain is more susceptible to boron deficiency than the Ferry strain, and consequently the results of the two seasons are to be compared with that fact in mind.[8] In 1940 only sugar beets were planted on these plots. Seed was sown on May 25 in 1938, on June 20 in 1939, and on July 5 in 1940.

Stand counts of sugar beet and garden beet were taken on all plots in 1938 and 1939. No significant decrease in stand in any of the treated plots was noted. While there was a slight increase in stand in many of the treated plots in each year the average increase was not statistically significant for any treatment. No toxic effect of any of the treatments was evident in either year except for slight marginal chlorosis on the lower leaves of an occasional bean plant in the 60-pound-per-acre application of borax. This was decidedly a transient effect and there was no evidence of permanent suppression or reduction in yield.

The occurrence of heart rot in sugar beet on these plots in 1938, 1939, and 1940 is recorded in table 2. On the untreated plots the plants with heart rot of the tops averaged 18 to 56 percent. The 100-pound treatment with manganese sulfate did not alter the incidence of heart rot although it stimulated top growth perceptibly in some plots. The application of 20 pounds of borax per acre with or without the incorporation of manganese or other minor elements was sufficient to eliminate heart rot completely in 1938. Moreover, the carry-over effect of the borax treatments in 1939 was sufficient to prevent the disease in that year. In 1940 the 40- and 60-pound treatments made 3

[7] The writers are indebted to Dr. J. H. Torrie, Department of Agronomy, University of Wisconsin, for advice in the arrangement of these and other plots discussed in this paper and in the statistical analysis and interpretation of results.

[8] The Morse strain of Detroit Dark Red is considered by Magruder et al. (*15*) to be so different from the original type that they refer to it as a distinct variety, under the name Morse Detroit.

years previously were still effective while those made 2 years before were all effective. However, in the 20-pound-per-acre treatment of 1938 the available boron was so low in 1940 that some disease developed. It may be concluded that in this soil, boron was retained in available form for three crop seasons, but that in the third season it was again becoming insufficient for normal development of sugar beets at the 20-pound level, through depletion, leaching, or fixation in an unavailable form.

TABLE 2.—*The effect of broadcast applications of borax and of salts of certain other minor elements on the incidence of heart rot in sugar beets grown in boron-deficient Poygan silty clay loam near Winneconne, Wis., in 1938, 1939, and 1940*

Treatment		Plants showing heart rot (average of 4 replicates) in—				
		1938	1939		1940	
Borax per acre	Manganese sulfate per acre	Treated in 1938 only	Treated in 1938 only	Treatment repeated in 1939	Treated in 1938 only	Treatment repeated in 1939
Pounds	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
[1] 0	0	18	25	34	50	56
0	0	28	26	35	23	30
0	100	28	47	31	34	35
20	0	0	0	0	2	0
20	50	0	0	0	0	0
20	[2] 50	0	0	0	9	0
40	0	0	0	0	0	0
40	50	0	0	0	0.5	0
60	0	0	0	0	0	0

[1] No fertilizer was applied in this plot; in all other plots 3–12–12 fertilizer was added at the rate of 450 pounds per acre in 1938, and 300 pounds in 1939 and 1940.
[2] There was also added per acre in this plot: 50 pounds $CuSO_4.5H_2O$, 50 pounds $ZnSO_4.7H_2O$, 50 pounds $FeSO_4.7H_2O$, and 100 pounds NaCl.

The parallel results in 1938 and 1939 with red beets are given in table 3. In order to study the progress of diseases, three sets of random samples were taken from each plot in 1938 at approximate 2-week intervals, beginning on July 29. There was with both varieties a decided increase both in percentage of disease and in disease index from July 29 to August 15, but there was little change from August 15 to August 31. Throughout the data the severity of the disease as indicated by the disease index was correlated closely with the percentage of diseased plants. The disease was reduced significantly in all treatments containing borax as compared with the untreated plots. It is to be noted that although heart rot of sugar beet was completely eliminated by the borax treatments, internal black spot of garden beet was present to some degree in most of the treatments. It is thus evident that the latter type is more susceptible to boron deficiency than the former and that Good For All, as already shown in the sand-nutrient studies, is more susceptible than Detroit Dark Red (Ferry strain). Although there was a trend toward less disease with increase in the rate of borax applied, the differences between borax treatments were not usually significant. The 100-pound treatment with manganese sulfate, as with sugar beets, had no effect upon internal black spot, although it did perceptibly stimulate growth. In the 20-pound borax treatment the disease was increased when 50 pounds per acre of manganese sulfate was added. This may have been due to the fact that growth was stimulated somewhat

TABLE 3.—*The effect of broadcast applications of borax and of salts of certain other minor elements on the incidence (percentage) and severity (disease index) of internal black spot in garden beets grown in boron-deficient Poygan silty clay loam near Winneconne, Wis., in 1938 and 1939* [1]

Treatment		Good For All variety in—										Detroit Dark Red variety [2] in—									
		1938: Notes on—						1939: Notes on Aug. 13				1938: Notes on—						1939: Notes on Aug. 13			
		July 29		Aug. 15		Aug. 31		No further Treatment		Treatment repeated		July 29		Aug. 15		Aug. 31		No further treatment		Treatment repeated	
Borax (Pounds per acre)	Manganese sulfate (Pounds per acre)	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index
		Percent		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*	
0 [3]	0	17	4.7	51	21.3	70	35.0	73	58.4	68	51.4	8	2.6	12	5.5	19	7.9	77	65.0	73	56.2
0	0	29	12.7	54	32.0	56	23.9	59	48.7	68	52.5	15	4.6	55	25.4	44	13.0	68	47.0	86	79.8
0	100	22	6.6	62	35.1	63	27.7	50	42.1	68	43.2	10	3.9	40	20.5	44	17.4	56	50.5	33	25.5
20	0	2	.3	16	3.2	20	5.8	15	8.4	5	2.8	1	.2	9	2.4	6	1.9	16	11.4	4	2.2
20	50	2	.3	22	5.9	22	8.4	10	6.1	14	6.1	4	1.9	14	4.2	8	3.4	18	12.6	11	6.7
20	50 [4]	7	1.8	19	6.9	22	8.8	40	20.8	9	4.7	3	1.0	8	1.6	10	2.9	19	9.0	11	4.8
40	0	0	.0	12	2.3	18	5.7	9	4.6	2	1.3	0	.0	1	.2	5	1.2	7	1.8	0	.0
40	50	2	.8	6	1.1	21	3.6	4	1.5	7	2.5	0	.0	3	.5	7	2.1	12	5.8	1	.2
60	0	0	.0	6	1.3	15	3.7	4	1.5	2	.6	0	.0	9	4.0	4	.8	3	1.8	2	.9
Difference required for significance (19:1):																					
Between nonborax treatments		28	12.2	29	17.9	27	11.4	32	37.9	17.0	17.8	15	5.5	29	15.3	27	12.9	51	58.0	47	46.4
Between borax treatments		[5]	[5]	16	6.2	19	5.8	18	11.9	7.0	3.5	[5]	[5]	12	4.6	7	3.0	14	11.2	10	6.6

[1] The percentages and indices in the table are averages from random samples in each of 4 replicates.
[2] Ferry strain used in 1938; Morse strain used in 1939.
[3] No fertilizer was applied in this plot; in all other plots 3-12-12 fertilizer was applied at the rate of 450 pounds per acre in 1938 and 300 pounds per acre in 1939.
[4] There were also added per acre: 50 pounds $CuSO_4.5H_2O$, 50 pounds $ZnSO_4.7H_2O$, 50 pounds $FeSO_4.7H_2O$, and 100 pounds NaCl.
[5] Since the presence of a number of treated plots with no diseased beets would introduce a bias in the statistical analysis of the data, the minimum significant difference was not calculated.

by the manganese, and presumably the need for boron was correspondingly increased. At the 40-pound-per-acre borax treatment the manganese treatment did not affect the disease reading, possibly because there was a more nearly adequate supply of boron available. At the 20-pound level the addition of salts of several other minor elements with the manganese sulfate did not affect the disease readings.

It may be concluded from this experiment that the internal black spot of garden beet was greatly reduced by application of borax and that the effect was carried over to the second year in this soil, inasmuch as the readings in 1939 for Good For All were essentially the same in plots receiving no further treatment as in those treated again in that year. In Detroit Dark Red most plots showed higher disease readings in 1939 than in 1938. This is undoubtedly due in part to the variation in susceptibility of the two strains of that variety. It emphasizes the importance of care in the selection of the seed stock used in conducting studies of boron deficiency.

It is also important to compare the results of garden beet with those of sugar beet. In the latter, heart rot was completely controlled by 20 pounds of borax per acre, while in the former 60 pounds did not completely correct black spot. It should, of course, be recognized that the comparison is concerned with the symptoms in the leaves of one plant and the symptoms in the roots of the other. A fairer basis might have been had if the roots of sugar beet had been cut and examined also.

Detailed notes of top symptoms on garden beet were taken. They are not presented in detail here, but it is worthy of mention that while top symptoms seemed as a rule to appear slightly in advance of root symptoms, they were, nevertheless, not necessarily concomitant with them. An appreciable percentage of roots showed black spot without recognizable top symptoms, and vice versa. It is possible that symptoms in the tops sometimes appear during a temporary shortage of boron and that normal growth is resumed before macroscopic root symptoms occur.

During the course of these experiments soil samples from representative areas were taken and analyses for available boron conducted by Berger and Truog (*3*). Their results showed a rather close correlation between available boron and the occurrence of black spot n this particular series of plots.

Side-of-row application.—In 1938 a series of plots was laid out in which borax and the salts of other minor elements were applied as side bands. These materials were mixed with the fertilizer and placed in bands at both sides of and 1½ inches away from the row and at the same depth as the seed. The results are given in table 4. None of the treatments was injurious to the stand. There was no significant difference between the percentages of diseased plants in the plots treated with any of the materials, except borax, and the untreated plots. Zinc sulfate appeared to reduce the disease index significantly but the percentage of diseased plants was nearly as high as that of the control. Since this material was used in combination with the salts of other minor elements in many other experiments without any effect upon the disease, this result is not considered significant. The 15-pound treatment with borax was not very beneficial, but beginning with 25 pounds per acre, the disease was reduced consistently

as the amount of this material increased. It was thus evident, as in the broadcast treatments, that boron was the only one of the elements used which had a beneficial effect in controlling internal black spot. It was also shown that under the conditions of these experiments the results were of the same order when the material was applied in concentrated bands at the sides of the row as when it was applied broadcast. The stand was not injured when as much as 80 pounds of borax per acre was applied in this manner. While this heaviest treatment practically eliminated the trouble, there was still an occasional diseased root found, as was the case when 60 pounds per acre was applied broadcast.

TABLE 4.—*The effect of salts of various minor elements on internal black spot of Detroit Dark Red garden beet (Ferry strain) when applied in bands at both sides of the row at time of sowing, Winneconne, Wis., 1938* [1]

Material applied	Amount applied [2] (per acre)	Stand of plants (per yard)	Disease development	
			Plants diseased	Disease index
	Pounds	*Number*	*Percent*	
None		13.4	36.0	22.4
Copper sulfate	50	11.1	46.3	27.0
Ferric sulfate	50	15.6	32.6	11.4
Zinc sulfate	25	13.6	30.6	7.6
Cobalt chloride	20	14.1	45.0	22.8
Manganese sulfate	50	13.9	33.6	10.2
(3)	(3)	16.4	58.5	25.1
Borax	15	14.5	[4] 32.6	6.8
Do	25	13.5	13.6	6.8
Do	35	15.5	11.7	3.6
Do	45	13.9	10.2	3.5
Do	55	13.9	1.6	1.2
Do	65	14.5	2.4	.8
Do	80	12.6	.5	.3
Difference required for significance (19:1):				
Between borax treatments			[4] 8.1	4.0
Between nonborax treatments			30.7	13.3
Between all treatments		5.3		

[1] Seed was sown on May 25; harvest data taken on Aug. 25.
[2] Calculated on the basis of 15 inches between rows.
[3] 50 lbs. $CuSO_4.5H_2O$, 50 pounds; $MnSO_4.4H_2O$, 50 pounds; $FeSO_4.7H_2O$, 25 pounds; $ZnSO_4.7H_2O$, 20 pounds, $CoCl_2.6H_2O$, and 100 pounds NaCl per acre were applied.
[4] The results from the 15-pound-per-acre treatment were not included in the analysis because the variance within this treatment was significantly greater than that within the other treatments.

FIELD EXPERIMENTS AT CLYMAN, WIS.

The treatments conducted at Clyman in 1938 were on Clyde silt loam with a reaction of pH 7.0 to 7.5. They were laid out on a somewhat more extensive scale than those at Winneconne and were designed to provide data on yield as well as on disease development. The beets grown on the area used in 1938 had only a negligible amount of disease, and the results were therefore confined to yield. A split-block experimental design was used in which treatments were randomized within varieties. Three replicates were employed. Plots consisting of four rows each were planted with a standard four-row beet drill equipped with an attachment by which the fertilizer and borax or other materials were applied in a band in a separate furrow 1½ inches away from and of the same depth as that in which the seed was placed. Two varieties were used, Detroit Dark Red (Ferry strain) and Good For All. Fertilizer (3–12–12) was applied in all plots at the rate of 150 pounds per acre.

The results of 1938 are given in table 5. It is to be noted that the stand was not injured in any instance, and in the case of Good For All

TABLE 5.—*The effect of borax and salts of certain other minor elements applied along the row at sowing*[1] *on the stand and yield of garden beets grown on Clyde silt loam at Clyman, Wis., 1938*[2]

Application of borax (pounds per acre)	Detroit Dark Red variety[3]		Good For All variety	
	Stand per yard	Yield per acre	Stand per yard	Yield per acre
	Plants	*Tons*	*Plants*	*Tons*
None	14.3	16.3	16.8	13.2
10	16.5	17.5	17.8	13.7
20	16.3	19.0	19.0	13.6
30	18.6	18.5	24.2	15.6
40	14.9	20.0	21.7	15.9
60	14.7	21.0	21.0	14.8
(4)	15.0	18.8	21.9	14.1
Difference required for significance (19:1)	3.4	1.5	3.7	1.5

[1] Borax applied in a furrow 1 to 2 inches deep and 1½ inches away from the row.
[2] Seed sown May 20; harvest data, taken Sept. 22, was from 3 replicates.
[3] Ferry strain secured from Ferry-Morse Seed Co., Detroit, Mich.
[4] A mixture of 50 pounds $CuSO_4.5H_2O$, 50 pounds $MnSO_4.4H_2O$, 50 pounds $FeSO_4.7H_2O$, 25 pounds $ZnSO_4.7H_2O$ and 10 pounds $CoCl_2.6H_2O$ per acre was applied.

it was increased significantly, in the three heaviest treatments with borax and in the treatment with minor-element salts other than borax. It is also to be noted that in the case of Detroit Dark Red the yield was increased significantly in all treatments except that of 10 pounds of borax per acre, and in the case of Good For All in all except those of 10 and 20 pounds of borax per acre. This showed rather definitely that even where the boron naturally available in the soil was sufficient to prevent black spot under the growing conditions of that season, the growth of the plant was stimulated sufficiently to produce significant increases in yield. This type of stimulation was not confined to borax since one or more of the materials applied in the mixture of salts of other minor elements also brought significant increases in yield. Since severe black spot occurred in this field in 1937, it is possible that the boron supply was marginal but that growing conditions in 1938 were such that enough boron was available to prevent macroscopic necrosis, though still not sufficient for optimum growth.

The experiments at Clyman in 1939 were laid out with the same plot design in another field of the same type of soil having a reaction of about pH 7.5. Because of the interest in the comparative value of applying borax with the fertilizer in bands or broadcast, the two methods were compared at 20-, 40-, and 100-pound-per-acre levels. The results are given in table 6. A significant reduction in black spot is noted when each treatment is compared with the control.

The disease was greatly reduced in all cases by borax. As in the trials at Winneconne, the percentage of diseased plants and the severity of disease tended to be reduced with increasing amounts of borax, but even at 100 pounds per acre there were still appreciable amounts present in each variety and the differences between treatments were not always significant. The stand was not materially affected by either type of treatment nor by any level of borax. The two methods of treatment had, as a rule, closely similar effects.

The yields were taken with only one variety, Detroit Dark Red. Although the crop was not so heavy as that at Clyman in 1938, the yields were greater in all treated plots than in the untreated ones. In each of five treatments the difference was greater than that required for significance, while in the other two treatments the difference was nearly great enough to be significant.

TABLE 6.—*The effect of application of borax, broadcast and at the side of the row, on the stand, yield, and internal black spot of garden beet grown on Clyde silt loam near Clyman, Wis., 1939* [1]

Application of borax		Detroit dark red variety [2]				Good for all variety		
Amount (pounds per acre)	Method	Stand per yard	Disease		Yield per acre	Stand per yard	Disease	
			Plants affected	Severity			Plants affected	Severity
		Plants	*Percent*	*Disease index*	*Tons*	*Plants*	*Percent*	*Disease index*
None		14.3	39.6	27.3	6.1	8.2	64.9	51.6
20	Side of row	15.0	10.7	8.1	7.7	7.8	19.1	10.8
20	Broadcast	12.9	18.7	11.1	7.1	7.4	18.5	9.4
40	Side of row	14.8	16.0	11.3	6.9	8.8	14.5	8.4
40	Broadcast	14.7	14.7	10.0	7.2	6.9	14.5	8.6
60	Side of row	14.5	10.7	6.9	8.2	9.0	10.5	3.8
100	do	16.3	10.7	6.7	8.1	10.6	4.6	2.3
100	Broadcast	16.4	5.3	2.9	7.8	7.5	7.2	3.4
Difference required for significance (19:1)		3.8	9.6	7.0	1.1	2.2	7.0	6.1

[1] Seed sown May 25; harvest Oct. 5.
[2] Ferry strain.

FIELD EXPERIMENTS AT ROCKFIELD, WIS.

The experiments at Winneconne and at Clyman were on relatively heavy clay loam or silt loam soils which were rather high in organic matter. No evidence of plant injury or yield depression was noted even with applications as high as 100 pounds of borax per acre. In 1939 a field of Miami silt loam was chosen near Rockfield, Wis., where black spot had been observed on previous crops and in which the soil was somewhat lighter in texture. This was laid out and planted on the same plan as the experiment at Clyman in 1939, in which side-of-row and broadcast applications of various amounts of borax were compared as to their effect on stand, disease incidence, and yield. As shown in table 7, there was neither reduction nor increase of stand as a result of the applications, and no significant effect upon yield. The disease developed relatively late in the season, but by the time of harvest a considerable amount was present in the untreated plots. In this experiment the disease was less in the 20-pound treatment than in the untreated plots, but the decrease was barely significant in the side-of-row application. In this case at the threshold treatment it would appear that the broadcast method was definitely better than the side-of-row method. However, a larger body of data would be needed to demonstrate the point in view of the fact that the difference was not consistent at higher applications in this series nor in the Clyman experiment of 1939. There was less disease at 30, 40, and 60 pounds per acre than at 20 pounds but the differences between the three higher treatments were not consistent with the amount of borax applied nor were they statistically significant. The lowest disease

incidence and index were at 100 pounds per acre applied broadcast but again this heavy treatment did not completely control the disease.

TABLE 7.—*The effect of application of borax, broadcast and at the side of the row, on stand, yield, and internal black spot of garden beet grown on Miami silt loam near Rockfield, Wis., 1939; Detroit Dark Red variety, Ferry strain*

Application of borax		Stand per yard	Disease		Yield per acre
Amount (pounds per acre)	Method		Plants affected	Severity	
		Plants	*Percent*	*Disease index*	*Tons*
None		[1] 19	38.8	28.0	11.3
20	Side of row	20	27.7	18.2	11.1
20	Broadcast	17	[1] 17.0	[1] 7.9	8.9
30	Side of row	21	11.0	5.3	11.4
40	do	17	10.7	4.0	8.8
40	Broadcast	16	14.2	5.8	11.1
60	Side of row	20	15.9	8.1	10.9
100	do	16	9.9	4.4	10.0
100	Broadcast	21	1.4	1.1	10.5
Difference required for significance (19:1)		[2]	18.7	11.3	[2]

[1] Each value is the average of 2 replicates.
[2] Differences not significant.

Application of Borax During the Growing Season

It was observed repeatedly that the symptoms of boron deficiency in both garden and sugar beets usually appeared about midseason. In the data from Winneconne in 1938 (table 4) it was shown that the disease increased rapidly during the first half of August. The question arose as to whether if borax were applied at or slightly before the first appearance of symptoms most of the damage to the crop would be prevented, and whether application at such a time, when the plant was in immediate need of boron, would be more effective than treatment at the time of planting. To obtain information on these points experiments were conducted at Winneconne in 1938, 1939, and 1941. In 1938 seed of Detroit Dark Red (Ferry strain) garden beet was sown on May 25, borax was applied in a trench at the side of the row on July 16, and harvest was completed on September 28. In 1939 Detroit Dark Red (Morse strain) garden beets were sown on June 20, borax in a water suspension was applied over the row on August 3, and harvest was completed on September 14. In 1941 sugar beets were sown on June 7, borax was applied in dry form broadcast on July 14, and the crop was harvested on October 6. In 1938 and 1939 top symptoms of boron deficiency were not apparent at the time of treatment, but they appeared in the controls about a week after treatment. In 1941 a trace of heart rot was present at the time of treatment. The results of these treatments are given in table 8.

Midseason treatments were very effective in all cases. In 1938 the results were about the same as those in the nearby spring applications reported in table 4. In 1939 very effective control was secured, particularly with the 40-, 60-, and 80-pound treatments. The heart rot of sugar beet was reduced greatly in 1941 by a 20-pound treatment after disease symptoms had appeared, and the heart rot was completely eliminated by heavier applications. It is also important to note that in 1941 substantial and significant increases in yield were

brought about by the delayed treatment. There was an increase of approximately 70 percent over the untreated plots in the 40-pound treatment. The decrease in the 60-pound treatment as compared with the 40-pound application was due principally to a reduction in stand caused by rhizoctonia root rot.

TABLE 8.—*The effect of a single dressing of borax during the growing season on the occurrence of internal black spot of Detroit Dark Red garden beets and of heart rot of sugar beets grown at Winneconne, Wis.*

Amount of borax applied (pounds per acre)	Detroit Dark Red garden beets				Sugar beets	
	1938 [1]		1939 [2]		1941 [3]	
	Plants diseased	Severity of disease	Plants diseased	Severity of disease	Heart rot	Yield per acre
	Percent	*Index*	*Percent*	*Index*	*Percent*	*Tons*
0	45.5	26.4	91.3	83.7	75.4	7.2
20	16.0	10.4	8.7	5.0	6.5	9.5
40	5.5	1.7	2.0	1.4	0	12.2
60			0	0	0	9.6
80	11.5	3.8	.7	.7		
Difference required for significance (19:1)	9.8	8.3				1.6

[1] Data based on 50-plant samples taken at random from each of 2 replicates.
[2] Data based on 50-plant samples taken at random from each of 3 replicates.
[3] Data based on two 50-foot rows from each of 4 replicates.

In 1939 at Winneconne a single midseason treatment was compared with treatments in which two and three applications were made at intervals of approximately 1 week. The results of this series are given in table 9. The disease was reduced to a small amount by a single 40-pound treatment. One supplementary 20-pound treatment reduced it still more while two subsequent 20-pound treatments completely eliminated black spot. This would indicate that the lack of complete control experienced in previous treatments at planting time or in midseason may be due either to lack of sufficiently complete distribution of boron in the soil or to lack of complete availability throughout the growing period. The greatest need for boron under average Wisconsin summer conditions for garden beets and sugar beets appears to be during the period immediately following the midpoint between sowing and harvest. In this experiment the first application of borax was made 44 days after sowing and 42 days before harvest.

TABLE 9.—*The effect of one, two, and three top dressings of borax during the growing season on the occurrence of internal black spot in Detroit Dark Red beets (Morse strain) sown at Winneconne, Wis., on June 20, 1939*

Amount of borax added per acre on dates indicated			Disease development [1]	
Aug. 3	Aug. 9	Aug. 16	Plants diseased	Severity of disease
Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
0	0	0	88.9	78.9
40	0	0	1.3	1.3
40	20	0	.6	.6
40	20	20	0	0

[1] Data from 50-plant samples taken at random from each of 3 replicates on September 14.

In 1940 and 1941 the method of midseason application, i.e., broadcast dry or applied as a spray in water suspension, was compared at Clyman with application at planting time. In 1940, since only a trace of black spot appeared, no data on disease control were secured. Yield records showed no consistent difference between the midseason spray treatment and side-dress treatment at planting at 20-, 40-, and 60-pound applications of borax. The midseason dry treatment showed a depression in yield at each level of borax when compared with the other two treatments. The differences were barely significant, however. The results in 1941 are given in table 10. The midseason spray was slightly better than the broadcast treatment at planting time in both disease control and in yield, while the midseason dry treatment was definitely less effective in both respects. It is therefore evident that midseason treatments are more effective if sufficient water is applied with the borax to facilitate immediate availability to the plants.

TABLE 10.—*Effect of borax applied by different methods on the incidence and severity of black spot and on yield of Perfected Detroit garden beets grown on Clyde silt loam at Clyman, Wis., 1941* [1]

Borax applied			Disease development		Yield per acre
Amount (pounds per acre)	Method	Time	Plants diseased [2]	Severity of disease [2]	
			Percent	*Disease index*	*Tons*
None			40	19.5	4.4
50	Broadcast	Planting time	5	1.2	4.6
50	Spray	Midseason	3	.9	5.0
50	Dry	Midseason	7	4.5	4.4

[1] Seed sown May 27; harvest data taken Aug. 19.
[2] Based on 25-beet samples from each of 3 replicates.

Effect of Application of Borax on Canning Quality of Garden Beets

Samples were taken from the plots at Clyman for canning tests in 1938, 1939, and 1940 to determine whether boron applications had any detrimental effects on canning quality. In 1938 composite samples from the three replicates of each treatment were tested for the two varieties used. In 1939 and 1940 separate samples were taken from each replicate of plots receiving no borax, and those receiving 20, 40, 60, and 100 pounds per acre. No significant differences in quality were found between the samples from treated and untreated plots when examined by several canners who judged the canned product.

Relation of Liming of Soil to Internal Black Spot

The common occurrence of boron deficiency in garden beet in soils neutral or alkaline in reaction has already been noted. Overliming of soils has been associated with boron deficiency by numerous investigators. Presumably boron becomes less available to the plant in an alkaline soil, although the nature of its fixation is not fully understood. Cook and Millar (*8*), Muhr (*17*), Wolf (*26*), Ferguson and Wright (*13*), and Midgley and Dunklee (*16*) are of the opinion that boron is fixed in limed soils in the form of insoluble boron compounds, while Bobko

TABLE 11.—*The occurrence and extent of internal black spot in garden beets grown in 1940 on soils adjusted to various reaction levels after treatment with lime or sulfur in the spring of 1935*

Soil	Material applied in spring of 1935	Rate per acre	Soil reaction [1]							Internal black spot in—			
			Before treatment	1935	1936	1937	1938	1939	1940	Good For All		Detroit Dark Red	
										Plants diseased	Amount of disease	Plants diseased	Amount of disease
		Pounds	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*Percent*	*Index*	*Percent*	*Index*
Antigo silt loam	Sulfur	3,000	6.1	4.1	4.3	4.8	4.5	4.8	4.8	0	0	0	0
	do	2,000	6.0	4.4	4.8	5.4	5.4	5.3	5.3	0	0	0	0
	do	1,000	6.0	5.5	5.3	5.5	5.5	6.1	5.8	0	0	0	0
	None		6.1	6.0	6.0	5.8	6.3	6.2	6.3	0	0	0	0
	Calcium hydrate	3,000	6.3	6.8	6.2	6.4	6.7	7.6	6.5	0	0	0	0
	do	[2] 6,000	5.9	7.5	7.5	7.3	7.4	7.7	7.5	4	1	0	0
	do	[2] 9,000	6.1	8.0	7.8	7.5	7.9	7.8	8.0	40	27	2	1
Plainfield sand	Sulfur	700	5.0	4.5	4.6	4.1	4.5	4.5	4.3	[5]	[5]	[5]	[5]
	None		5.2	5.0	4.6	4.8	4.7	4.7	4.6	[5]	[5]	[5]	[5]
	Calcium carbonate	1,000	4.9	5.4	5.0	5.5	5.3	5.0	4.5	0	0	2	1
	do	3,000	4.9	5.3	5.7	6.0	5.4	5.5	4.9	14	7	6	3
	do	5,000	5.3	5.5	5.4	5.4	6.0	5.5	5.2	26	24	4	2
	do	7,000	5.2	6.0	6.5	6.6	6.5	6.5	6.3	22	12	6	1
	do	[4] 9,000	5.3	6.1	6.5	7.0	6.8	7.0	6.7	40	27	4	1

[1] Except for the sampling in the spring of 1935 before treatment, the reaction tests were made from samples collected in September of each year. Reaction tests were made by Dr. Harold H. Hull, Department of Soils.
[2] Plus 1,000 pounds calcium hydrate added in spring of 1936.
[3] Plus 2,000 pounds sodium carbonate added in spring of 1936.
[4] Plus 3,000 pounds calcium carbonate added in spring of 1936.
[5] No plants developed beyond the seedling stage.

et al. (*4*), Naftel (*18*), and Hanna and Purvis (*14*) are of the opinion that activity of soil micro-organisms accounts for some of the fixation.

In 1940 a study was made of the development of internal black spot in Good For All and Detroit Dark Red (Ferry strain) beets on two series of plots which had been treated with various amounts of lime or sulfur in the spring of 1935 to adjust the soil reaction to various levels. One series was located on Plainfield sand in central Wisconsin (near Arnott, Portage County); the other was on Antigo silt loam in northcentral Wisconsin (near Antigo, Langlade County). Each of the series was designed to provide a range of reactions from about pH 4.5 to 8.0. It may be seen from the annual pH readings in table 11 just how the reactions became adjusted and the extent to which they reverted to the original level during the 5-year period. Beets planted on these plots in the sixth season after treatment (1940) were examined for black spot (table 11).

In Antigo silt loam no black spot was found except in those treatments in which the soil reaction was still alkaline, and in the more susceptible of the two varieties the disease was decidedly more severe where the heavier treatment with lime had maintained the highest alkalinity. In Plainfield sand, however, the disease was marked at the 3,000-pound treatment, increasing with heavier dosages of lime. This correlation prevailed in spite of the fact that at no time did the soil reaction reach neutrality except in the heaviest treatment and that in two treatments the reaction had receded to around pH 5.5 at least 2 years before that in which beets were grown. It seems quite evident that in this light soil the liming treatment tied up boron without neutralizing the soil solution and that some of the boron remained unavailable to plants over a period of several years. This soil was so low in calcium that beets grew very poorly in the untreated and sulfur-treated plots, which were extremely acid, while the 1,000-pound-lime-treated plot, although its reaction was equally acid, supported good growth. This raises the question whether borax should not be applied generally on this soil in order to offset the fixation of available boron when it is limed for legumes or other crops.

DISCUSSION

The occurrence of internal black spot in garden beets is much more important than the relative percentage of affected plants would signify. This is because the necrotic areas are detected with difficulty and because their presence in the canned product even in small amounts has been ruled by food inspection authorities as sufficient to render a given lot unfit for sale as human food. Thus, a relatively small amount is enough to cause a complete loss. The initial purpose in the study with garden beets was to determine the relation of boron to the development of internal black spot, to learn whether any other minor element had a supplementary influence, and to ascertain how effective applications of borax to deficient soils could be for the control of the disease.

From the results presented in this paper there is abundant evidence that boron deficiency in the soil is responsible for the nonparasitic abnormal development of the garden beet which leads to necrosis in the root. It is also shown that varieties of garden beet vary in their pathologic response to this deficiency. Furthermore, table beets are in general more susceptible to this disease than sugar beets.

In the experiments conducted, the application of borax to the soil, either as a side-of-row band or broadcast, has never failed to reduce the amount of disease. However, as a rule, increasing amounts of borax, although they have tended to reduce the amount of black spot, have failed to control the disease completely. When substantial amounts of manganese, zinc, copper, cobalt, and sodium were applied no consistent change in the extent of black spot resulted. This seems to remove the probability of black spot being associated with a deficiency of other trace elements now regarded as essential at some time or other in the normal development of most crop plants.

It has become apparent in the studies in sand culture, in pot cultures with soil, and in midseason applications of borax in the field that boron when first applied as borax or boric acid is readily available through roots or leaves and is transported rapidly to those meristematic regions where its deficit becomes most quickly apparent. It is equally apparent that boron rather readily becomes locked in an immobile form in the tissues of maturing organs or regions within such organs, and once in that state it is of little immediate use to the meristematic tissues. This observation is in agreement with the recent work of Wolf (*26*) with cauliflower.

Since the quality requirements of table beets make it important that meristematic regions in the root have adequate boron at all times, the program of control must take into account the availability of boron in the soil and its mobility in the plant. The consistent lack of complete control when large quantities of borax were applied at planting time has not been adequately explained. It is apparent from 4 years' observation that the disease develops in late July or August in Wisconsin, when the crop is usually about midway between sowing and harvest. At this period 40 to 60 days have elapsed after the application of borax. When borax was applied in midseason before the appearance of symptoms the disease was controlled as well as and in some cases better than when applied at the time of planting.

In the experiments on silty clay loam at Winneconne about the same degree of control was secured in the second year as in the season immediately following spring application, indicating that a considerable part of the boron applied remained available in this alkaline soil in addition to that which may have leached away or become fixed chemically or biologically. On the other hand, it was obvious from the experiments on sandy loam at Arnott that treatments with lime tended to result in less available boron 5 years later and long after the soil reaction had become quite acid.

The most acute and severe development of black spot has occurred when a protracted dry spell during which the plants have slowed down very decidedly in growth is followed by abundant soil moisture and a sudden increase in growth. Under such conditions the plants seem to be unable to get adequate boron for all their needs and necrotic break-down occurs. If borax has been applied to the soil most plants secure enough boron, but some fail to do so and a certain amount of disease results. Some indication of this may be seen in the work of Eaton and Wilcox (*12*) on boron toxicity in soils of various types under various conditions. They point out that—

> When new boron is added to a soil * * * drying is an important adjunct to fixation. When soils are dried, the concentrations of all soil-solution constituents are increased to saturation, which is followed by precipitation. In the soils with-

out added boron this precipitation is reversible, since on rewetting, the initia concentrations are again found. When boron is added to the soil and then dried, however, a part of that which has been added is not recovered on resuspension.

Variation between individual plants and between strains of garden beets is another factor which probably enters into the clinical picture of the black spot disease. It has been shown that Good For All is very susceptible and that the two strains of Detroit Dark Red differ in their reaction under some field conditions. It would be of interest to inquire further into the nature of these differences. The nature and extent of the root system might have an important bearing upon the reaction of individuals or strains in the acquisition of boron from the soil when it is needed most vitally by the plant.

SUMMARY

The development of the symptoms of boron deficiency on young seedlings of garden beet in quartz-sand culture is described. The disease appeared promptly as an intensification of red pigment, unilateral development of leaf lamina, stunting, and death of the growing point. In plants allowed to grow slightly older before boron deficiency became acute, symptoms of the same type appeared more slowly. Boron became fixed promptly in the tissues, with the result that mature leaves did not yield mobile boron readily to meristematic regions. However, soluble boron as boric acid in the nutrient supplied through the roots or applied to older leaves was transported readily to the aerial growing tip, stimulating normal growth. In garden beets grown in complete solution long enough for taproot enlargement, typical black spot appeared gradually after the boron supply was eliminated, in rings in which bundles had differentiated most recently. Plants grown in the greenhouse on soil from fields where severe black spot of garden beet or heart rot of sugar beet had occurred ordinarily did not show acute or severe symptoms, although growth responses to the addition of borax to the soil were secured. Greenhouse diagnosis of boron deficiency by pot tests was not satisfactory.

In a survey of the occurrence of internal black spot of garden beet in Wisconsin during the growing seasons of 1937, 1938, 1939, and 1940, the disease was found most often in soils with neutral or alkaline reaction. It occurred on soils ranging from light sandy loam to dark silty clay loam and was about equally prevalent in high and low parts of the field.

In field experiments at several locations in Wisconsin during the seasons of 1938, 1939, 1940, and 1941 the application of borax to the soil consistently reduced the internal black spot disease of garden beet and the heart rot disease of sugar beet.

Other minor elements, including manganese, zinc, copper, iron, and cobalt, when applied as soluble salts, did not reduce the disease.

Heart rot of sugar beet was corrected effectively with smaller applications of borax than were necessary for the control of internal black spot of garden beet. Even as heavy applications as 100 pounds of borax per acre often did not completely eliminate the black spot disease.

Broadcast applications on slightly alkaline Poygan silty clay loam in 1938 were effective for two successive seasons and partly so for the third season. During the first season broadcast applications of as

high as 60 pounds per acre, disked into this soil prior to planting, were not injurious to either garden or sugar beet and only very slightly so to the garden bean, which is very sensitive to boron toxicity.

Applications of borax with the fertilizer in bands 1½ to 2 inches removed from and at the same depth as the seed were equal in effectiveness to broadcast treatments.

Increases in yield of garden beet commonly, but not always, resulted from borax applications. An increase occurred in one instance where the boron naturally available in the soil was sufficient to prevent black spot.

Midseason applications of borax, either in the dry form or in liquid suspension, were effective in controlling black spot when carried out just before or at the time of the first appearance of symptoms in the leaves.

Applications of borax up to 100 pounds per acre did not alter the canning quality of the garden beet.

Boron deficiency appeared in garden beets grown on Antigo silt loam limed 5 years earlier to maintain an alkaline reaction during the intervening period. Where the lime applied had not brought the reaction up to neutrality black spot did not occur. In Plainfield sand similarly treated, however, black spot appeared in all limed plots and severely in one in which a pH reading as high as 6.0 had been recorded in only 1 of the intervening 5 years. This shows that soil types differed in the degree to which available boron was tied up by liming and that, in sandy soil, boron deficiency was made acute by liming without the soil solution approaching an alkaline reaction and for a 5-year period after the treatment.

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THE NUTRITIVE VALUE OF CERTAIN FISH MEALS AS DETERMINED IN TESTS WITH SWINE AND RATS[1]

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INTRODUCTION

The use of fish meal in mixed protein supplements for fattening swine has become widespread in certain sections of the Corn Belt. The value of this practice was demonstrated by Vestal[2] at the Purdue Experiment Station.

As several different kinds of fish meals are available, there is a demand for information on their comparative feeding value. Menhaden meal from the east coast and sardine and herring meals from the west coast are the most readily available at prices the swine feeder is willing to pay.

The purpose of this investigation was to determine whether there is any significant difference in the nutritive value of several commercial fish meals when fed to swine and rats.

LITERATURE REVIEW

Numerous investigations have been conducted with chickens and white rats which show that the nutritive value of fish meals may vary as a result of source of material and method of processing. It has repeatedly been demonstrated (*1, 2, 3, 5, 10, 11, 12, 13, 14, 16, 17, 18*)[3] that vacuum-dried meals and meals dried at reduced temperatures are superior in feeding value to those dried at high temperatures. White fish meals have frequently given better results than dark fish meals, although the variety of fish from which the meal is made appears to have much less effect on the finished product than the freshness of the material and the method of processing.

The temperature used in the drying process is one of the most important causes of variation in the finished product. The consistently high feeding value reported for vacuum-dried meals as compared to flame-dried meals indicates the necessity of controlled temperature during the drying process. Ingvaldsen (*6*) found that when meals were subjected to temperatures higher than 190° C., a diminution in arginine and cystine occurred. He also reported (*7*) lowered tyrosine, tryptophane, and cystine content of fish meals due to putrefaction.

Few experiments to determine the comparative value of different kinds of fish meal in swine rations have been reported. Investigators at the Iowa Agricultural Experiment Station (*8*), Foster and Hostetler (*4*), Kyzer and Jones (*9*), and Willman and Morrison (*19*) found very

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[2] See VESTAL, C. M. MIMEOGRAPHED REPORTS, 1937, 1938, 1939, 1940.

[3] Italic numbers in parentheses refer to Literature Cited, p. 133.

little difference in the feeding value of different fish meals for fattening swine, particularly when the meals were fed in combination with other protein feeds.

MATERIALS AND METHODS

Feeding trials were conducted with both swine and white rats. Swine were used for comparative trials of the feeding value of flame-dried menhaden, sardine, and herring fish meal in a commonly used mixed protein supplement. Rats were used to determine the protein efficiency of the fish meals when they were fed as the only protein supplement to corn. Two vacuum-dried whitefish meals and a steam-dried sardine meal were also tested under these conditions. The rate and efficiency of gain were used as a basis for evaluating the results. The results were treated statistically by the analysis of variance technique as given by Snedecor (*15*). Significant mean differences at the 5-percent level and highly significant mean differences at the 1-percent level are given when such differences existed.

SWINE-FEEDING TRIALS

Three similar feeding trials with the fish meals were carried out with swine in dry lot. The first trial was conducted during the winter of 1939–40, the second during the summer of 1940, and the third during the winter of 1940–41.

EXPERIMENTAL ANIMALS AND RATIONS

The pigs were obtained from the Purdue experimental swine herd of Duroc-Jerseys. All the animals were healthy and normal in size for their age at the beginning of the experiment. In each trial the pigs were divided as uniformly as possible according to size, sex, and probable ability to gain in the feed lot. The average weight of the pigs was approximately 70 pounds each at the beginning of the experiment, and they were fed until they reached a market weight of approximately 225 pounds. Ten pigs were started in each lot in the first trial, 15 in the second, and 12 in the third. One pig in the lot, fed sardine meal, died in the second trial, and 1 pig was removed from the sardine feed-lot in the third trial after 69 days. The quarters for each lot of pigs consisted of a colony house and a dry lot approximately 30 feet wide and 60 feet deep.

The rations were composed of No. 2 yellow shelled corn fed free choice with a protein supplemental mixture composed of 40 percent expeller-process soybean oil meal, 20 percent meat and bone scraps, 20 percent fish meal, 10 percent cottonseed meal, and 10 percent alfalfa leaf meal. A mineral mixture containing 10 pounds of steamed bonemeal, 10 pounds of pulverized limestone, and 1 pound of common salt was available to the hogs at all times. Block salt was also kept before the hogs. The shelled corn, mixed protein supplement, mineral mixture, and salt were fed free choice in self-feeders.

The experimental rations differed only in the kind of fish meal included in the mixed protein supplement. The other constituents of the protein supplement were obtained on the local market. A complete chemical analysis was made of each ingredient of the mixed supplement (table 1).

TABLE 1.—*Analysis of the feeds used in the mixed protein supplement of the swine rations*

Ingredient	Trial No.	Moisture	Protein	Fat	Fiber	Ash	Nitrogen-free extract
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Menhaden fish meal (flame-dried)	1	5.7	66.2	4.4	0.3	17.5	5.9
	2 and 3	6.4	65.8	5.1	.5	18.3	3.9
Sardine fish meal (flame-dried)	1 and 2	5.1	70.9	5.5	.4	11.4	6.7
	3	6.7	65.1	4.3	.8	16.9	6.2
Herring fish meal (flame-dried)	1 and 2	5.1	75.1	6.6	.4	11.1	1.7
	3	8.2	69.9	9.2	.8	11.5	.4
Soybean oil meal	1	7.7	45.4	6.8	5.6	5.0	29.5
	2 and 3	8.7	46.1	5.5	6.1	4.7	28.9
Meat and bone scraps	1	5.0	51.0	7.6	2.4	30.1	3.9
	2 and 3	5.7	49.9	6.2	2.0	34.2	2.0
Cottonseed meal	1	6.2	41.1	6.2	9.9	6.3	30.3
	2 and 3	4.8	41.3	5.8	10.0	6.9	31.2
Alfalfa leaf meal	1	6.8	18.2	2.9	20.4	9.1	42.6
	2 and 3	4.3	24.9	3.6	16.2	8.2	42.8

The initial weight recorded for each pig is the average of weights taken on 3 consecutive days. The 3-day average weight was considered the starting weight. A similar procedure was followed in determining the final weight at the close of the experiments. Group weights were taken every 10 days and individual weights every 30 days. The feed records were tabulated by 10-day periods corresponding to the 10-day weight periods.

EXPERIMENTAL RESULTS

Because of the difference in protetin content of the fish meals there was a slight variation in the total protein of the supplements containing the different fish meals. This variation was not more than 2.4 percent in any of the trials.

The results of the three feeding trials are shown in table 2. The

TABLE 2.—*Weights, gains, and feed consumption of pigs fed different kinds of fish meal* [1]

[Summary of 3 trials]

Fish meal in ration	Trial No.	Hogs fed	Period on feed	Average initial weight	Average final weight	Average total gain	Average daily gain	Average daily feed consumption	Total feed per 100 pounds of gain	Ratio of consumption of protein supplement to corn
		Number	*Days*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
Menhaden (flame-dried).	1	10	90	71.1	222.7	151.6	1.68	6.6	383	1:7.9
	2	15	85	69.0	205.0	136.0	1.60	5.9	368	1:6.0
	3	12	100	57.8	233.3	175.5	1.76	7.1	400	1:9.5
	Average	12.3	91.7	66.0	219.0	153.0	1.67	6.4	384	1:7.6
Sardine (flame-dried).	1	10	90	71.0	228.7	157.7	1.75	6.4	364	1:5.4
	2	14	85	69.0	205.4	136.4	1.60	5.9	366	1:7.7
	3	11	100	58.2	238.4	180.2	1.80	7.1	396	1:10.3
	Average	11.7	91.7	66.1	222.4	156.3	1.71	6.4	376	1:7.6
Herring (flame-dried).	1	10	90	71.5	215.2	143.7	1.60	6.3	393	1:5.8
	2	15	85	69.0	206.5	137.5	1.62	6.0	370	1:7.2
	3	12	100	57.9	230.8	172.9	1.73	6.8	394	1:10.2
	Average	12.3	91.7	66.1	216.8	150.7	1.64	6.3	385	1:7.6

[1] Rations self-fed, free choice: Corn, fish-meal supplement mixture, and mineral.

differences in gains between the lots were not statistically significant in any of the trials.

In trial 1, conducted during the winter of 1939–40, the rate of gain was 1.68, 1.75, and 1.60 pounds respectively for the menhaden-, sardine-, and herring-fed lots. The group receiving herring meal required 392.7 pounds of feed for each 100 pounds of gain, while the lot fed menhaden meal required 383.3 pounds and the lot fed sardine meal required only 363.9 pounds. The lots fed sardine and herring meal both consumed excessive amounts of the protein supplement.

In trial 2, conducted in the summer of 1940, for all three lots the rates of gain and of feed consumption for each 100 pounds of gain were practically the same; however, in this trial the lot receiving the menhaden meal consumed more supplement than the lots receiving sardine and herring meals. This may have been due to the fact that a fresh supply of menhaden meal was used in this trial.

The third trial, conducted in the winter of 1940–41, confirmed the results of the previous trials. There was very little difference in either rate of gain or feed consumption for the three lots.

SUMMARY OF RESULTS

The results with swine indicate that the beneficial qualities of fish meal as a constituent of mixed supplements were supplied equally well by menhaden, sardine, and herring meals. However, there was a slight tendency for the gains to be a trifle higher on the sardine fish-meal ration and the feed required for 100 pounds gain was also slightly less. The results also show that the same commercial brand of fish meal may vary considerably in palatability as judged by a comparison of feed consumption.

RAT—FEEDING TRIALS

The feeding experiments with rats consisted of two parts. The first was designed to gain information on a larger number of fish meals than was studied with hogs. The consumption of feed was unrestricted and fish meal was used as the only protein supplement to corn. The second was planned to obtain a more accurate comparison of the protein efficiency of the fish meals used in the swine-feeding trials by equalized feeding of groups of three animals.

FEED CONSUMPTION UNRESTRICTED

In the "ad libitum" experiments, six different fish meals were compared in two similar trials. The mixed rations consisted of finely ground yellow corn with sufficient fish meal added to bring the protein content to approximately 14 percent. One percent of cod-liver oil and 2 percent of a mineral mixture were included in the rations. The composition of the rations is shown in table 3 and the analysis of the fish meals in table 4.

TABLE 3.—*Rations fed to rats to determine the comparative value of different fish meals*[1]

Feed used	Ration number					
	1	2	3	4	5	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Yellow corn	87.2	87.2	87.2	87.2	87.2	86.5
Menhaden fish meal (flame-dried)	9.1					
Sardine fish meal (flame-dried)		8.5				
Sardine fish meal (steam-dried)			8.5			
Herring fish meal (flame-dried)				8.0		
Whitefish meal (vacuum-dried)					9.3	
Whitefish meal blend (vacuum-dried)						10.5
Dextrin	.7	1.3	1.3	1.8	.5	0.0
Mineral [2]	2.0	2.0	2.0	2.0	2.0	2.0
Cod-liver oil	1.0	1.0	1.0	1.0	1.0	1.0
Total protein	14.03	14.10	14.05	13.91	14.02	13.89

[1] Feed consumption unrestricted.
[2] The mineral mixture was composed of 10 parts limestone, 10 parts special steamed bonemeal, and 1 part common salt.

TABLE 4.—*Analysis of fish meals used in rat experiments*

Fish meal in ration	Moisture	Protein	Fat	Fiber	Nitrogen-free extract	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Menhaden (flame-dried)	5.7	66.2	4.4	0.3	5.9	17.5
Sardine (flame-dried)	5.1	70.9	5.5	.4	6.7	11.4
Sardine (steam-dried)	5.5	71.0	3.4	.6	3.8	15.7
Herring (flame-dried)	5.1	75.1	6.6	.4	1.7	11.1
Whitefish meal (vacuum-dried)	13.4	64.1	3.6	.4	0	20.9
Whitefish meal blend (vacuum-dried)	6.5	57.2	7.5	2.2	3.2	23.4

Corn supplied 57.9 percent of the total protein in the ration and fish meal 42.3 percent. When necessary, dextrin was added to balance the ingredients. The corn used was No. 2 yellow dent, and the fish meals were standard commercial grades.

Albino rats were started on feed in individual cages at about 3 weeks of age, when they weighed 35 to 45 gm. each, and were fed for a period of 8 weeks. Weekly records were kept of the gains made and the feed consumed. Ten litters of rats were used, one animal from each litter being represented on each ration. In the first trial there were six males and four females on each ration.

The results of the two trials are shown in table 5. The differences in gain in the first trial were not significant; however, there was a

TABLE 5.—*The nutritive value of different fish meals in rations for rats* [1]

Fish meal in ration	Trial No.	Mean gain	Adjusted mean gain	Mean feed consumption	Feed per gram of gain
		Grams	*Grams*	*Grams*	*Grams*
Menhaden (flame-dried)	1	185.9	199.1	777.9	4.19
	2	192.6	200.1	715.9	3.72
Sardine (flame-dried)	1	211.6	197.9	851.3	4.02
	2	243.2	211.5	826.8	3.40
Sardine (steam-dried)	1	188.5	197.3	788.6	4.18
	2	208.3	199.3	762.7	3.66
Herring (flame-dried)	1	177.7	204.4	741.2	4.17
	2	170.5	215.4	610.0	3.58
Whitefish meal (vacuum-dried)	1	198.8	185.0	851.7	4.28
	2	205.3	191.8	775.2	3.78
Whitefish meal blend (vacuum-dried)	1	196.2	172.8	879.7	4.48
	2	190.0	191.8	732.1	3.85

[1] Feed consumption unrestricted.

significant difference in the amount of feed consumed, indicating that the fish meals varied in palatability. In order to adjust the mean gains to the same level of food consumption the data were analyzed by the analysis of covariance (table 5). A difference in the adjusted mean gain of 15 gm. is significant at the 5-percent level and a difference of 20 gm. is significant at the 1-percent level. The following significant differences in the nutritive value of the fish meals are shown: Herring higher than whitefish and whitefish blend; sardine higher than whitefish blend; sardine (steam-dried) higher than whitefish blend; and menhaden higher than whitefish blend.

In the second trial eight males and two females were fed on each ration. As in the previous trial each rat had a litter mate on every other ration.

The analysis of the data of the second trial shows there was a significant difference in the gains made as well as in the amount of feed consumed. The least significant mean differences were 32.8 gm. for the mean gain and 83.6 gm. for the mean feed consumption. The data show that the flame-dried sardine, steam-dried sardine, and whitefish meal all produced significantly larger gains than the herring meal. However, the feed consumption for the herring meal group was significantly lower than for any other ration. This may have been due in part to its high fat content.

In order to determine whether the differences in gain resulted entirely from variation in quantity of feed consumed or from a difference in the nutritive value of the fish meals, the mean gains were adjusted for feed consumption as in trial 1. The results (table 5) demonstrate that palatability does not account for all the difference in gain. A difference of 14.5 gm. gain is significant at the 5-percent level and a difference of 19.4 gm. is significant at the 1-percent level.

An interesting result revealed by this analysis was that the herring meal was apparently the least palatable and produced the smallest gain, yet it appeared to have a higher nutritive value than some of the other fish meals. The results of the controlled feeding trails substantiate this conclusion.

CONTROLLED FEEDING

The menhaden, sardine, and herring fish meals were compared by applying the paired feeding technique to triplicates. Each member of a triplicate received the same amount of feed, the member consuming the smallest amount limiting the intake of the other two. A triplicate was composed of litter mates of the same sex that did not vary more than 3 gm. in starting weight.

These trials were conducted with rations of approximately 8.5, 10.5, and 12.5 percent protein (table 6). The proportion of protein supplied by the corn and fish meal was kept the same at each level. The feed was weighed out to each rat daily to the nearest 0.1 gm. on a torsion balance and the gain in weight of the rats was recorded by 7-day periods. Eight rats were fed on each fish meal at each protein level for a period of 7 weeks.

A summary of the results for the three protein levels is shown in table 7. The least significant mean differences in gain were 3.85, 3.47, and 4.01 for the 12.5-, 10.5-, and 8.5-percent levels of protein respectively. The data indicate that there were significant differ-

ences in the nutritive value of the meals. Except at the 8.5-percent protein level the sardine meal showed a higher nutritive value than the herring and menhaden meals, and the herring meal showed a higher nutritive value in all three trials than the menhaden. At none of the levels of protein intake was the difference in the gains made on the sardine and herring meals significant at the 1-percent level. However, at all three levels of protein intake, both the sardine and herring meals produced gains that were significant at the 1-percent level over those produced by the menhaden meal. This is also shown by the means of the three levels for each fish meal (table 7, last column) where a difference of 2.47 gm. is necessary for the 5-percent level and a difference of 3.31 gm. for the 1-percent level.

TABLE 6.—*Rations fed to rats in controlled feeding tests of different fish meal rations*

Feed used	Trial 1 rations (at the 12.5-percent protein level)			Trial 2 rations (at the 10.5-percent protein level)			Trial 3 rations (at the 8.5-percent protein level)		
	1	2	3	1	2	3	1	2	3
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Precent*
Yellow corn	80.0	80.0	80.0	67.6	67.6	67.6	55.3	55.3	55.3
Menhaden fish meal	8.3			7.1			5.8		
Sardine fish meal		7.7			6.6			5.4	
Herring fish meal			7.3			6.2			5.1
Dextrin	8.7	9.3	9.7	22.3	22.8	23.2	35.9	36.3	36.6
Mineral	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cod-liver oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total protein	12.31	12.44	12.24	10.72	10.60	10.59	8.63	8.61	8.66

TABLE 7.—*The nutritive value of different fish meals in rat rations at three levels of protein intake* [1]

Fish meal in ration	Rats at each protein level	At the 12.5-percent protein level				At the 10.5-percent protein level				At the 8.5-percent protein level				Mean gain on each fish meal [2]
		Average initial weight	Average final weight	Average total gain	Average feed consumed	Average initial weight	Average final weight	Average total gain	Average feed consumed	Average initial weight	Average final weight	Average total gain	Average feed consumed	
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Menhaden	8	37.50	149.50	112.00	414	35.75	145.50	109.75	436	36.10	88.35	52.25	290	91.33
Sardine	8	37.75	161.87	124.12	414	35.50	153.75	118.25	436	36.00	92.12	56.12	290	99.50
Herring	8	37.75	157.37	119.62	414	35.75	149.75	114.00	436	36.10	94.10	58.00	290	97.21

[1] Feed consumption restricted.
[2] Least significant difference at the 5-percent level-2.472 gm.; least significant difference at the 1-percent level-3.307 gm.

The data obtained in this experiment in which feed consumption was controlled confirm the results shown by the analysis of covariance in the ad libitum feeding trials; namely, that the herring meal had a nutritive value nearly as high as the sardine meal although it was much less palatable. In the paired feeding trials the rats receiving herring meal most consistently limited the feed intake of the other members of the triplicate. The menhaden meal was second in this respect.

DISCUSSION OF RESULTS

The rat-feeding experiments showed that the fish meals differed in palatability and nutritive value. However, these differences were not detectable in swine-feeding trials when the fish meals were fed in combination with several sources of protein.

The whitefish meals (vacuum-dried) used in this investigation were not found to be superior to flame-dried menhaden meal as has been reported in the literature (*3, 12, 14, 16*).

It is realized that additional work in this field of investigation is necessary before definite conclusions can be formed, for the same kind of fish meal may vary considerably from time to time because of uncontrollable conditions inherent in the fish-meal industry. The results of this investigation should therefore not be applied to all commercial fish meals until they are verified by testing a larger number of samples of the same kind of meals.

SUMMARY AND CONCLUSIONS

A study was made of the comparative nutritive value of several different commercial fish meals by including them in rations of swine and rats.

Menhaden, sardine, and herring fish meals were found to have equal value in a mixed protein supplement when fed to swine.

The experiments with rats showed differences in palatability and nutritive value of the fish meals when they were fed as the only protein supplement to corn.

Ad libitum feeding experiments with rats showed that the sardine and whitefish meals were more palatable than the menhaden and herring meals. Sardine meal appeared to be slightly superior to the other meals in palatability and nutritive value. Although herring meal was the least palatable it had a nutritive value comparable to sardine meal.

Controlled feeding trials with rats showed that the sardine and herring meals were superior in nutritive value to the menhaden meal.

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FUNGI ASSOCIATED WITH CERTAIN AMBROSIA BEETLES[1]

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INTRODUCTION

The association of certain fungi wth the tunnels of ambrosia beetles has been recognized for nearly 100 years (*1*).[3] These ambrosia fungi apparently are used as food by the beetles, and different fungi are cultivated by different species or groups of species (*2*). Recently, Leach et al. (*3*), in a paper on two ambrosia beetles and their associated fungi, summarized the past work on ambrosia fungi, pointing out particularly the scarcity of exact information on this group.

In 1937 the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, started a general study of the biology of ambrosia beetles of the Southern States, and the Division of Forest Pathology cooperated in studying the fungi associated with certain ambrosia beetles of economic importance in the deterioration of green hardwood logs and lumber. The present paper is a report of the mycological findings of this study.

MATERIALS AND METHODS

Wood infested with ambrosia beetles was furnished largely by H. R. Johnston, of the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, at Saucier, Miss. Some material was gathered at various sawmills in Louisiana. All the beetle material was identified by Johnston.

All cultural studies were made on malt agar (1½ percent agar and 2½ percent malt) at room temperature, approximately 75° to 85° F. Isolations were made by allowing live beetles to walk on the agar surface and also by making transplants from the wood adjacent to tunnels. Some cultures were purified by making single-spore transfers from the original cultures secured from the beetles or from wood. Numerous freehand and microtome sections were cut from wood blocks that contained insect tunnels, for the study of the fungi as they occur in nature.

The species of ambrosia beetles studied were *Platypus compositus* Say, *Pterocyclon mali* (Fitch), *P. fasciatum* (Say), *Xyleborus affinis* Eich., and *X. pecanis* Hopkins.

[1] Received for publication April 22, 1942. The Southern Forest Experiment Station of the Forest Service, U. S. Department of Agriculture, cooperated in these investigations by providing facilities for field studies at Saucier, Miss.

[2] The author is indebted to Ross W. Davidson, of the Division of Forest Pathology, for advice on the taxonomy of the fungi described; and to Edith K. Cash, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, for translating descriptions of species into Latin.

[3] Italic numbers in parentheses refer to Literature Cited, p. 144.

FUNGI ISOLATED FROM AMBROSIA BEETLES

WOOD-STAINING FUNGI

Although a great many fungi, bacteria, yeasts, and nematodes were isolated from ambrosia beetles and their tunnels, only the food fungi and general-staining fungi were given special attention. Stain accompanying ambrosia beetle attack is of two distinct types: (1) A restricted darkening adjacent to the tunnels, and (2) general staining of the type common in sapwood without beetle attack.

Restricted blackened areas are common, if not universal, around tunnels of *Platypus compositus*, *Pterocyclon mali*, and *P. fasciatum*

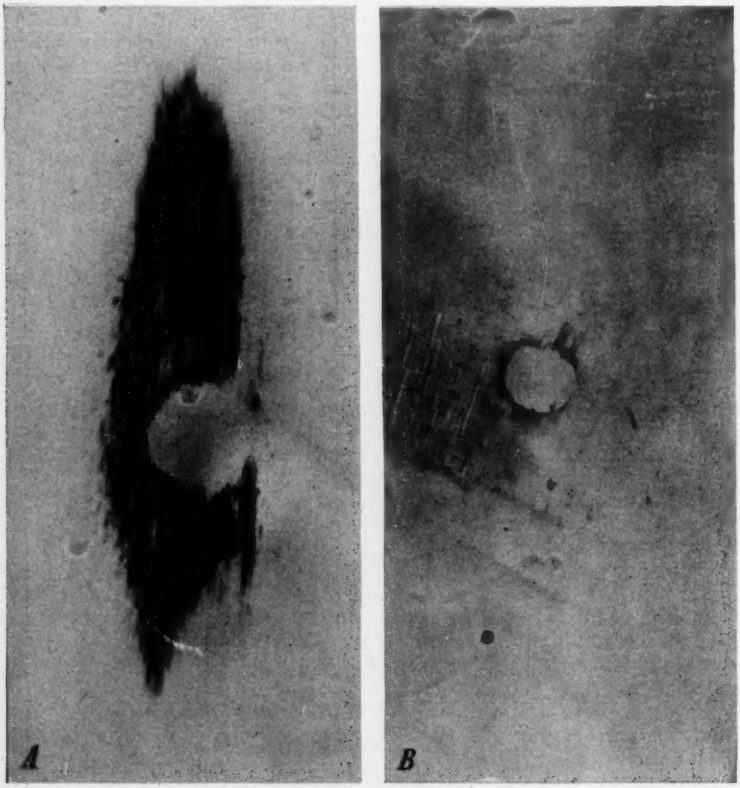

FIGURE 1.—Black stain around tunnels of two ambrosia beetles: *A*, *Platypus compositus*; *B*, *Pterocyclon mali*. × 15.

in sapwood but absent or indistinct in heartwood. Stain is commonly absent around the tunnels of *Xyleborus affinis*, but occasionally a light-brown stain is found around the tunnels of this species in sweetgum (*Liquidambar styraciflua* L.) and southern sweetbay (*Magnolia virginiana* L.). Around the tunnels of *Xyleborus pecanis* no black stain was observed, although some browning of the adjacent wood cells occurred. Black stain is most pronounced with *Platypus*

compositus (fig. 1, *A*), extending longitudinally from the tunnel as much as 5 mm., although laterally it spreads but a few cells from the edge of the tunnel. With *Pterocyclon* (fig. 1, *B*) the black is just as intense but usually extends less than 1 mm. from the tunnel.

Isolations from black stain around tunnels yielded a number of organisms, but the only ones consistently isolated were the ambrosia or food fungi of the beetles concerned; these fungi are described later in this paper. Microscopic examinations of the blackened areas showed the wood cells to be filled with fungus material of a deep-brown color, which in some cases could be identified as an ambrosia fungus by the presence of spores. When the surface of sapwood blocks of sweetgum and oak was sterilized for 30 seconds in boiling water, and the blocks were then inoculated with cultures of the ambrosia fungi, stain was produced. This stain, however, was not as intense as that found around the tunnels of *Platypus compositus* and *Pterocyclon* spp. Indications are that the stain around beetle tunnels is caused by the ambrosia fungi, although the color may be intensified by reactions of these fungi with insect secretions or other organisms, such as yeasts and bacteria, which are commonly present.

General staining of the type common in sapwood without beetle attack is not universal with ambrosa beetle attack. However, past tests and observations have shown that severe staining of logs is not prevented by chemical sprays when ambrosia beetle attack is heavy (*5*). The role of ambrosia beetles in disseminating general wood-staining fungi has been discussed more fully in another paper (*7*).

AMBROSIA OR FOOD FUNGI

Ambrosia Fungus of Platypus compositus

The only fungus consistently observed in the tunnels of *Platypus compositus* or consistently isolated from the adults or wood adjacent to the tunnels of this insect was a species of *Endomyces* Rees. A review of the literature disclosed no described species having the spore, asci, and hyphae measurements of this ambrosia fungus or any species of *Endomyces* with only two-spored asci. Therefore, the fungus is here described as a new species:

Endomyces bispora sp. nov.

On malt agar, colonies (fig. 2, *C*) are very slow growing, reaching 5–8 mm in radius in 6 days at room temperature (75° to 85° F.). At first the colony is hyaline, then turns a light yellowish brown, although under the microscope the hyphae and spores appear hyaline. The edge of the colony is distinctly mycelial with many conidia formed singly or in heads on short recumbent conidiophores or laterally on the hyphae (fig. 2, *A* and *B*). The cells of the conidiophores often disjoint and act as conidia, and buds form from the conidia. Most of the colony is composed of a slimy mass of conidia and ascospores, which give it a yeastlike appearance. Practically no aerial mycelium is formed.

Conidia commonly germinate on malt agar by producing buds, forming a yeastlike colony from the margin of which hyphae eventually protrude. No germination of ascospores was observed.

Conidia, including disjointed conidiophores and buds, are hyaline, unicellular, and of various shapes and sizes: 2.5μ–12.5μ×2.2μ–5.0μ, averaging 6.3μ×3.8μ, usually elongate and somewhat pear-shaped. Asci are hyaline, slightly pear-shaped, and are usually formed terminally in small groups of two to six. Asci are occasionally formed singly at the tip or nodes of the asci-bearing hyphae (fig. 2, *A*) or in compound heads on short branches. Asci are 7.5μ–11.2μ× 6.2μ–10.0μ, averaging 7.4μ wide and 8.6μ long. Asci-bearing hyphae range from 1.8μ to 3.3μ in diameter, averaging 2.5μ. Ascospores (fig. 2, *A*) are hyaline, hat-shaped, 6.2μ–9.8μ, averaging 7.2μ, across the brim, and 1.8μ–2.2μ, averaging 2.4μ,

are usually alined within the ascus with the brims extending from the base to the in width perpendicular to the brim. The brim surface is concave. The spores

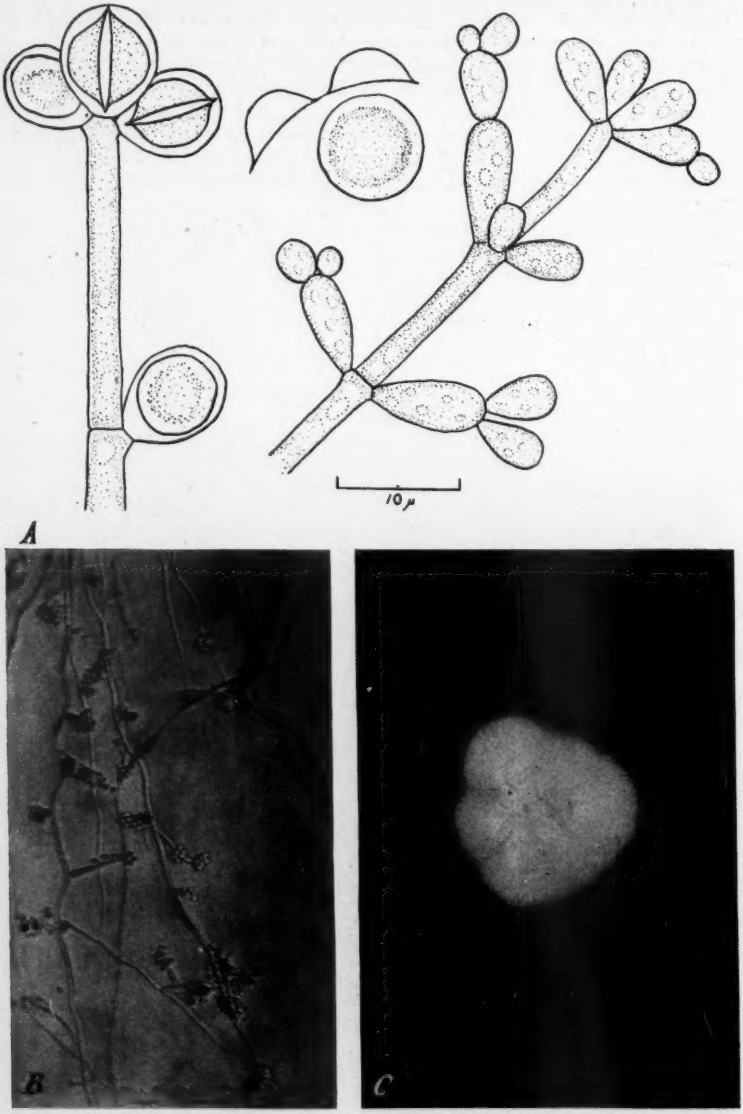

FIGURE 2.—*Endomyces bispora* on malt agar: *A*, Asci, ascospores, and conidia; *B*, margin of a culture showing hyphae and conidia production, × 150; *C*, culture 15 days old grown at about 80° F., × 1.

tip of the ascus and nearly filling the ascus. Only two spores were observed in each ascus.

Isolated from adults of *Platypus compositus* Say and from wood adjacent to their tunnels. Observed in pecan (*Carya* sp.), sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and locust (*Gleditsia* sp.).

Culturae in agaro maltoso lente crescentes, primum hyalinae, dein pallide flavo-brunnescentes, margine distincte myceliali; conidia numerosa, singula vel in capitulis in conidiophoris brevibus recumbentibus vel in lateribus hypharum formata; cellulae conidiophororum saepe disjungentes et ut conidia agerentes; gemmae e conidiis formatae; conidia conidiophoris et gemmis inclusis hyalina, unicellularia, 2.5μ–12.5μ longa, 2.2μ–5.0μ lata, plerumque elongata et paulo pyriformia; asci hyalini, paulo pyriformes, plerumque terminales et 2–6 caespitosi, interdum ad apicem vel nodos hypharum ascophorarum singuli, vel in capitulis compositis in ramis brevibus nati, 7.5μ–11.2μ longi, 6.2μ–10.0μ lati, bispori; ascosporae hyalinae, pileiformes costulatae, costula inculsa 6.2μ–9.8μ in diam., ad angulum rectum in costulam 1.8μ–3.2μ in diam., vulgo costulis e basi ad apicem extensis dispositae et ascum fere implentes.

The ambrosia in the insect tunnel consists of conidia and asci, at first hyaline, but, as larval activity increases, changing into a slimy yellowish coating on the tunnel wall. The adjoining wood-cell lumina become filled with hyphae and conidia, which turn to deep brown, causing an intense black stain of the wood adjacent to the tunnel. The reason for the darkening of hyphae and conidia in the wood was not determined, although inoculations showed that the color change occurred.

Ambrosia Fungus of Xyleborus affinis

An imperfect fungus, which apparently is the ambrosia of *Xyleborus affinis*, was observed in association with this beetle and was consistently isolated from tunnels and from adults. The fungus is apparently an undescribed species of *Cephalosporium* Corda.

Cephalosporium pallidum sp. nov.

On malt agar, colonies (fig. 3, *C*) are moderately slow growing, reaching 9 to 14 mm. in radius in 6 days at room temperature (75° to 85° F.). The margins are usually appressed and hyaline while the rest of the colony is covered with a thin layer of hyaline, fluffy aerial mycelium which often becomes appressed with age except for isolated tufts. Aerial mycelium may be entirely lacking. Occasionally a slight brownish tinge develops in parts of old cultures. Yellowish, yeasty mounds develop in aging cultures. In the yeasty mounds mycelium may be limited largely to pointed, short-celled hyphae (fig. 3, *A*) projecting but shortly from the yeasty mass of conidia and monilioid cells. Compact helicoid hyphal formations were commonly observed in the filamentous mycelium.

Conidia germinate on malt agar by forming monilioid chains of cells which finally give rise to hyphae (fig. 3, *A*). Spore heads are formed relatively soon after germination.

In culture typical fruiting consists of cephalosporic heads of conidia protruding but slightly above the agar (fig. 3, *A*) on erect or decumbent conidiophores. Conidiophores are usually unbranched and hyaline and terminate in 1 to 10 or more hyaline, unicellular conidia which are nearly spherical to slightly pear-shaped, 7.6μ–14.4μ long and 7.9μ–14.0μ wide, averaging 10.8μ×10.4μ. When appreciable aerial mycelium occurs, conidiophores elongate and branch (fig. 3, *A*). Sometimes conidiophores are composed partly or totally of moniliform cells, particularly in the yeasty mounds. Occasionally buds were observed forming laterally on hyphae (fig. 3, *A*), and monilioid chains of spores of irregular sizes and shapes were observed in the agar or protruding above it (fig. 3, *A*).

Isolated from adults of *Xyleborus affinis* Eich. and from the wood adjacent to their tunnels. Observed in sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and southern sweetbay (*Magnolia virginiana* L.).

Coloniae in agaro maltoso modice lente crescentes; mycelium plerumque appressum et hyalinum, in culturis vetustis brunneotinctum; conidiophora plerumque non ramosa, hyalina, in conidiis 1–10 vel pluribus, hyalinis, unicellularibus, fere sphericis vel leniter pyriformibus, 7.6μ–14.4μ longis, 7.9μ–14.0μ

latis terminata, partim vel omnino e cellulis moniliformibus composita; gemmae interdum in lateribus hypharum formatae; catenulae monilioideae sporarum magnitudine formaque variabilium praesentes; conidia in natura singula vel 2–4 in capitulis, rare in catenulis brevibus nata.

The ambrosia (fig. 3, *B*) in the tunnels of *Xyleborus affinis* consists of conidia, mostly solitary, sometimes in heads of 2 to 4 spores and occasionally in short monilioid chains. In new tunnels single

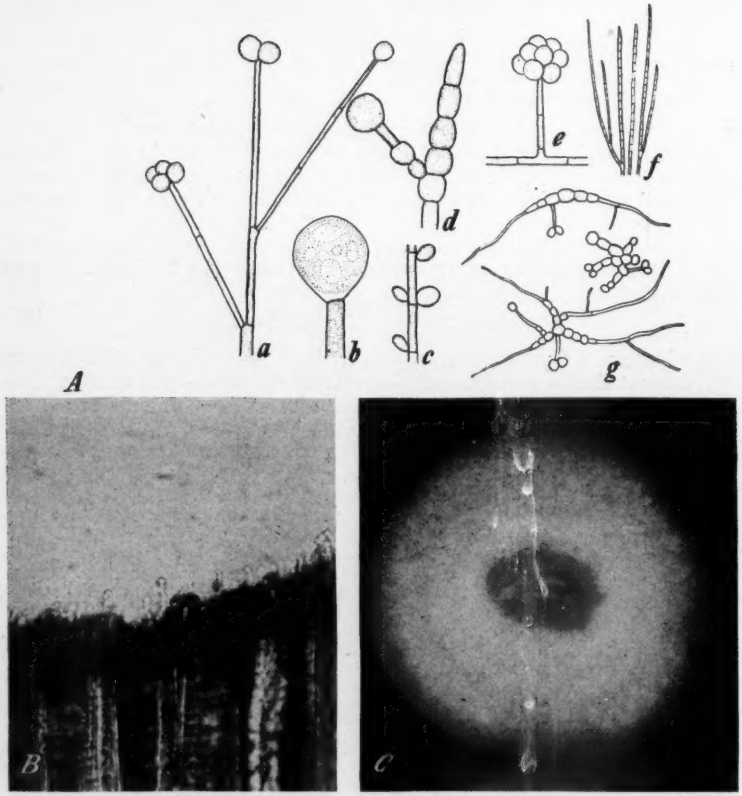

FIGURE 3.—*Cephalosporium pallidum*. *A*, Drawings from malt agar cultures: *a*, Branched conidiophore, × 320; *b*, single conidium, × 1,040; *c*, buds from hypha, × 320; *d*, monilioid cells, × 560; *e*, cephalosporic head, × 320; *f*, paraphyseslike hyphae, × 160; *g*, young colonies showing early production of conidia, × 120. *B*, Young ambrosia in insect tunnel, × 88. *C*, Culture 15 days old grown at about 80° F., × 0.8.

conidium-bearing conidiophores (12μ–38μ long) are easily seen, but later only a jumbled mass of cells is visible. The fungus mostly remains hyaline in the tunnel and adjacent wood cells, and does not produce stain around the tunnel except slightly in sweetgum and southern sweetbay.

The ambrosia fungus of *Xyleborus affinis* is apparently related to

that of *X. dispar*. The fungus associated with *X. dispar* was originally described by Hartig as *Monilia candida* (*1*). Neger (*4*) expressed the opinion that it may be an endomycete, although no asci or ascospores have been observed. Schneider-Orelli (*6*), in describing and illustrating the ambrosia fungus of *X. dispar*, shows it to be similar to that associated with *X. affinis* except that no mention is made of yellowish yeasty mounds or cephalosporic heads such as occur with the ambrosia fungus of *X. affinis*, although the author does illustrate a conidiophore bearing a single conidium similar to those in yeasty mounds of *Cephalosporium pallidum*. Furthermore, black stain occurs around the tunnels of *X. dispar* but is absent or not so pronounced around those of *X. affinis*.

Ambrosia Fungus of Xyleborus pecanis

The ambrosia fungus associated with the tunnels of *Xyleborus pecanis* is apparently an undescribed species of *Cephalosporium* Corda.

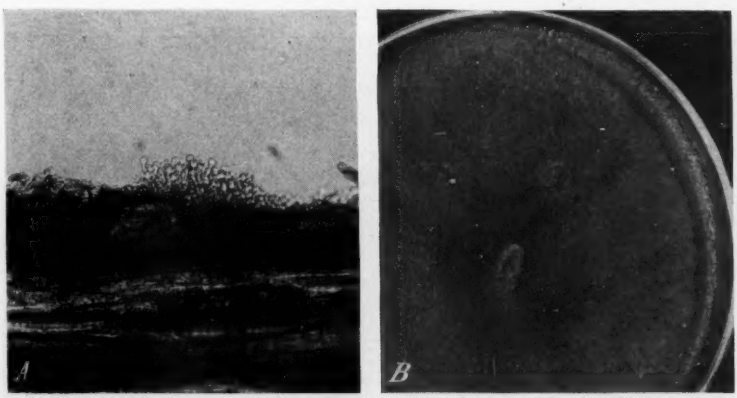

Fig. 4.—*Cephalosporium luteum:* *A*, Young ambrosia in insect tunnel, × 99; *B*, culture on malt agar, 15 days old, grown at about 80° F., × 0.8.

Cephalosporium luteum sp. nov.

On malt agar, colonies (fig. 4, *B*) are fast growing, reaching 60–70 mm. in radius in 6 days at 75° to 85° F. They are at first hyaline but soon become sulfur yellow to light brown. The agar is stained a deep brown. Aerial mycelium is at first fluffy but becomes appressed with age. No tendency for budding or yeasty growth was observed as with other ambrosia fungi encountered in this study. The margin of the colony is decidedly filamentous.

Fruiting on malt agar is sparse, and spores are often difficult to find. When a block of mycelium-bearing agar is removed from a culture and placed in a moist chamber, a few spores usually can be found along the cut edge on protruding conidiophores within 24 hours. Spores are formed on simple or branched conidiophores, mostly singly, sometimes in heads of two or three spores, are hyaline, nearly spherical, unicellular, and average 6.5μ in diameter. In nature spores are larger, 5.3μ–12.5μ wide and 6.1μ–15.0μ long, averaging 8.8μ×10.1μ.

Observed in association with *Xyleborus pecanis* Hopkins in swamp tupelo (*Nyssa biflora* Walter) and southern sweetbay (*Magnolia virginiana* L.).

Coloniae in agaro maltoso rapide crescentes, primum hyalinae, dein e sulphuris brunneolae; mycelium aereum primum floccosum, vetustum appressum; fructificatio in culturis sparsa; conidiophora plerumque non ramosa et brevia; sporae

singulae vel 2–3 in capitulis, hyalinae, fere sphericae, unicellulares, in culturis 6.5μ in diam., in natura 5.3μ–12.5μ latae, 6.1μ–15.0μ longae, plerumque singillatim interdum 2–3 in capitulis natae.

The ambrosia in the beetle gallery is at first hyaline but later forms a yellowish to brownish lining to the brood chamber. It consists of mycelium and conidia (fig. 4, *A*), mostly borne singly on short, simple or branched conidiophores, or in heads of two to three spores. The conidia remain hyaline. The wood around the chamber is stained a light brown.

Young ambrosia is similar to that in the tunnels of *Xyleborus affinis*. Later, however, the ambrosia of *X. pecanis* turns a sulfur yellow and does not become yeasty, as does that of *X. affinis*. The conidia of the ambrosia fungus of *X. pecanis* are, in general, similar to some of the conidia of the ambrosia fungi of *X. affinis* and of *X. dispar*, but no monilioid chains, such as occur in the last two, were observed. The ambrosia fungus of *X. pecanis* is apparently the same as that of *X. xylographus* Say or is closely related to it, as illustrated and described by Hubbard (*2*).

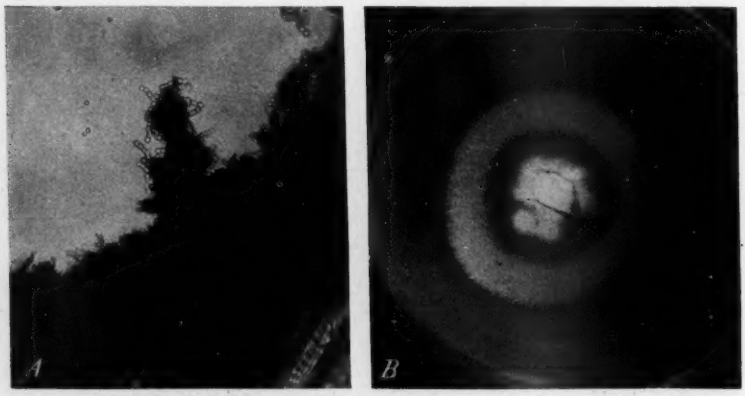

FIGURE 5.—*Monilia brunnea: A*, Ambrosia in insect tunnel, × 84; *B*, culture 15 days old grown on malt agar at about 80° F., × 0.7.

AMBROSIA FUNGUS OF PTEROCYCLON MALI AND P. FASCIATUM

The ambrosia fungus of *Pterocyclon mali* is apparently the same as the fungus associated with *P. fasciatum* and appears to be a new species of *Monilia* Pers.

Monilia brunnea sp. nov.

On malt agar, colonies are slow growing, reaching 8–10 mm. in radius in 6 days at 75° to 80° F. They are appressed, and at first are nearly hyaline but with age become dark brown (fig. 5, *B*). Original isolates were quite yeasty in general appearance and consisted largely of monilioid chains of rounded cells that budded in situ, similar to the growth described and illustrated by Leach et al. (*3*) for the ambrosia fungus of *Trypodendron betulae* Sw. and *T. retusum* (Lec.). After repeated subculturing, more filamentous mycelium was formed, although the monilioid type of growth persisted in parts of the culture. When filamentous mycelium was formed the surface of the culture became finely tomentose, the amount of aerial mycelium being limited. In old cultures there was a tendency

for small mounds to form, consisting mostly of monilioid cells. In one isolate short, pointed hyphae were formed, similar in shape to those described for the ambrosia of *Xyleborus affinis* but dark brown in color.

Monilioid cells are mostly hyaline, although in old cultures and old tunnels they may be distinctly brown, and are borne in simple or branched chains. Cells contain large granular particles. The terminal one to three cells are usually nearly spherical while the other cells are oblong or rectangular to spherical. Mature cells are 7.5μ–11.5μ long by 5.2μ–10.0μ wide, averaging 8.5μ in each direction. In some isolates there was a tendency in culture for conidia to collect in heads as well as in chains.

Spores germinate on malt agar by forming monilioid groups of cells that eventually give rise to hyphae.

Observed in association with *Pterocyclon mali* (Fitch) in sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), maple (*Acer* sp.), and oak (*Quercus* sp.), and with *P. fasciatum* (Say) in oak (*Quercus* sp.).

Culturae in agaro maltoso lente crescentes vetustae fuscescentes; mycelium plerumque appressum; sporae interdum gemmantes; hyphae in articulos oidiformes secendentes; sporae plerumque hyalinae, interdum in culturis vetustis et in viis insectorum brunneae, in catenulis simplicibus vel ramosis natae; sporae terminales 1–3 fere sphericae, alterae ex oblongis vel rectangularibus sphericae, 7.5μ–11.5μ longae, 5.2μ–10.0μ latae.

The ambrosia in the tunnels of the beetle consists of masses of monilioid cells (fig. 5, *A*) with little filamentous mycelium. Freshly formed ambrosia is hyaline, but in older tunnels it may turn dark brown. Ambrosia forms in both the main tunnel and the larval cradles. Wood cells adjacent to the tunnels become filled with hyphae and monilioid cells that soon turn brown, causing an intense black stain around the tunnel. Stain occurred in the wood of each species in which the beetles were found.

The ambrosia fungus of *Pterocyclon* apparently is closely related to that of *Trypodendron*. With both of these genera larvae are reared in separate cradles in contrast to the other genera encountered in this study, in which the larvae are free in the egg-laying tunnel. The ambrosia fungus associated with *Pterocyclon mali* and *P. fasciatum* apparently is a species of *Monilia* Pers. However, it is not identical with *Monilia candida* Hartig, associated with *Xyleborus dispar* (*6*), or the fungus described by Leach et al. (*3*) as the ambrosia of *Trypodendron betulae* and *T. retusum* and considered by those authors as a possible strain of *M. candida*.

SUMMARY

Four fungi commonly associated with southern species of ambrosia beetles are described as new species: *Endomyces bispora*, associated with *Platypus compositus; Cephalosporium pallidum*, with *Xyleborus affinis; C. luteum*, with *X. pecanis;* and *Monilia brunnea*, with *Pterocyclon mali* and *P. fasciatum*.

These fungi apparently are used as food by the ambrosia beetles with which they are associated and probably cause the restricted black or brown stain adjacent to the beetle tunnels.

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